

ASPECTS OF THE REPRODUCTIVE CYCLE OF  
ATLANTIC COD, *Gadus morhua* L., FROM  
INSHORE NEWFOUNDLAND

CENTRE FOR NEWFOUNDLAND STUDIES

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**Aspects of the reproductive cycle of Atlantic cod, *Gadus morhua* L.,  
from inshore Newfoundland.**

by

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## ABSTRACT

Data on maturity, condition and gonad histology were collected to give insight into the reproductive biology of Atlantic cod, *Gadus morhua* L., in Placentia and Trinity Bay, Newfoundland. Female cod in Placentia Bay spawned between April and August, with some spawning probably occurring later in the fall. Oocytes began to ripen for the next season 1-2 months after spawning with cortical alveoli appearing as early as July and exogenous vitellogenesis starting as early as August. Oocyte development continued throughout the winter. Spawning was usually determinate but the ability to boost fecundity by recruitment of oocytes into vitellogenesis during spawning was noted. Atresia of previtellogenic and vitellogenic oocytes existed as a mechanism to downgrade fecundity.

The testes of Placentia Bay cod were filled with spermatozoa between January and August and spermiation was likely possible over most of this period. Recovery from spermiation and the subsequent advance of the testes to spermiogenesis occurred quickly, probably over 2-4 months. Spermatogenesis was completed prior to the winter reduction in feeding intensity.

Seasonal changes in condition indices differed slightly for female and male cod from Placentia Bay. Female somatic condition factor increased from April right up until October as the fish fed and decreased throughout the winter, as oocyte development continued. The pattern was similar for males but maximum somatic condition factor was reached in August and then maintained until November, after which it decreased. Female

condition and gonadosomatic index was maximum during spawning. Male gonadosomatic index was also high immediately prior to and during spawning but there were two peaks in condition factor, one during spawning and one in November at the start of spermiogenesis.

The existence of non-reproductive cod suggests that spawning is not always annual for some individuals. In Smith Sound, Trinity Bay, over 30% of the female cod that had started to ripen were resorbing all developing oocytes by January and would have missed spawning in 1999. Spawning omission may have been triggered by food shortage during a critical period for gonad development. A small percentage of male cod had delayed gonad development but may still have been able to spawn in 1999. Data to support small percentages of cod with multiple year interruptions in oocyte development and ovarian senescence were also collected.

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## GENERAL INTRODUCTION

The Atlantic cod, *Gadus morhua* Linnaeus 1758, is a large demersal gadid distributed along the North American coast from Cape Hatteras to Ungava Bay (Cohen *et al.* 1990). The cod has traditionally supported the largest fishery in the northwest Atlantic, with catch totals during the 1960's reaching almost 2 000 000 tonnes annually (Lear 1993). Catches of cod decreased drastically during the early 90's and on July 2, 1992 a moratorium was placed on the Newfoundland and Labrador cod fishery.

The intent of the moratorium is to allow the cod stocks time to recover naturally to levels that could once again support a viable fishery. Overfishing and the selective removal of larger individuals from the population, however, has done more than just decrease biomass. Numerous aspects of the reproductive biology of this species have also changed, as summarized by Trippel (1995). These changes include decreases in the number and size of eggs produced, changes in spawning time and decreases in size and age at maturity. Such changes in reproductive biology may cause cod populations to have a lower reproductive capacity than in previous years and thus pose major problems to stock recovery.

Along with these changes, there appear to have been changes in the distribution of cod. Large schools of cod have been reported in the deep fjord-like inlets of eastern Newfoundland and have also been observed in unusually shallow water and near the surface. At the same time, off-shore catch totals are very low (Lilly 1996). Hutchings *et al.* (1993) believed that the occurrence of spawning cod inshore might indicate the

existence of coastal populations and suggested that since offshore stocks have collapsed, inshore cod may make a larger contribution to recruitment than has previously been believed. Lilly (1996) insisted that inshore spawning does not prove the existence of 'bay stocks'.

Other fish species have also experienced declines in biomass in recent years. Grand Banks capelin, *Mallotus villosus*, the principal prey of cod, declined from 7 million tonnes in 1990 to 0.1 tonnes in 1991 and 0.2 tonnes in 1992 (Miller 1992). It has been shown experimentally that reductions in food ration cause increases in atresia and thus decreases in fecundity (Scott 1962; Hester 1964; Bagenal 1969; Wootton 1973; Hislop *et al.* 1978; Kjesbu *et al.* 1991). Studies have also shown that poor condition, as a result of food shortage, can cause some species such as American plaice, *Hippoglossoides platessoides* (Pitt 1966) and winter flounder, *Pleuronectes americanus* (Burton & Idler 1984) to completely halt reproductive investment and undergo spawning omission. Accounts of post-mature non-reproductive cod are not plentiful but have been reported for the Barents Sea (Oganesyan 1993) and the Flemish Cap (Walsh *et al.* 1986) and there are suggestions of cod that may be skipping spawning in the fjord-like inlets of Trinity Bay (Bratney 1996). These reductions in reproductive investment could delay stock recovery since low spawner abundance (measured as number of spawners or number of eggs) leads to poor recruitment (Myers & Barrowman 1996).

There is a great need to obtain a better understanding of the reproductive development and potential of Atlantic cod if the intent of management strategies is to

rebuild northern cod stocks. The present study is a baseline examination of the reproductive biology of Atlantic cod from two inshore locations in Newfoundland; Placentia Bay and Smith Sound, Trinity Bay. Gonad maturity (visual and histological examination) and condition indices are monitored to determine the spawning period and the timing of the various stages in the development of both male and female gametes and the pattern of allocation of energy for gonad growth. The possibility of non-annual spawning is also explored.

## CHAPTER 1

### **Timing of reproductive development and details of the spawning pattern for female Atlantic cod, *Gadus morhua* L., from Placentia Bay, Newfoundland**

#### **1.1 ABSTRACT**

Reproductive status and condition indices for female Atlantic cod, *Gadus morhua* L., from Placentia Bay, Newfoundland were monitored between April 1998 and January 1999. Oocytes began to accumulate cortical alveoli in July and vitellogenesis was first observed in August. Spawning was recorded between April and August, during which time the gonadosomatic indices were high. Lowest gonadosomatic indices were recorded after spawning and a 1-2 month period followed spawning during which no oocyte development was apparent. Post-ovulatory follicles persisted for up to nine months after spawning. The collection of females in January that were in spent condition may indicate the existence of some fall spawning females in Placentia Bay.

The spawning pattern for this species appeared to be determinate in most cases but the ability to boost fecundity by *de novo* vitellogenesis during spawning was observed. Downgrading of fecundity was accomplished by follicular atresia. Evidence existed to suggest complete spawning omission by a small percentage of females in 1998, perhaps due to insufficient energy storage. The energy cycle was composed of a period of white muscle energy storage between April and October and the subsequent utilization of this energy during late fall and winter for metabolism and gonad growth.



## 1.2 INTRODUCTION

The fishery for Atlantic cod, *Gadus morhua* L., in the northwest Atlantic Ocean was placed under moratorium in 1992 after drastic declines in biomass estimates. Perhaps the decline of the northern cod stocks was in part due to a lack of understanding of the reproductive potential of this species. Trippel (1995) reported that before the closure of the fishery, it was not uncommon for 60 % of the older adult biomass in some areas to be removed annually, leaving the burden of producing future generations on smaller fish with lower reproductive capacities.

With hopes of rebuilding the collapsed northern cod stocks, researchers appear to have concentrated much of their efforts on understanding the reproductive biology of this species. These studies include reviews of present and historic spawning areas (Brander 1991; Hutchings *et al.* 1993; Gerasimova & Kiseleva 1995), changes in size and age at maturity (Trippel 1995; Morgan & Bratley 1996; Saborido-Rey & Junquera 1998) and changes in the timing of reproduction (Hutchings & Myers 1993a; 1993b). Other research has concentrated on the spawning strategy and the processes of gonad and gamete development. Morrison (1990) described changes in the macroscopic and microscopic appearance of cod gonads throughout the year. The late stages of reproductive development are best understood, due in part to advancements in aquaculture and the use of broodstock females. Kjesbu and Kryvi (1989) examined the cellular changes that occur during final oocyte maturation and details of spawning were examined by Kjesbu (1989), Chambers and Waiwood (1996) and Kjesbu *et al.* (1996). Kjesbu (1994) and Kjesbu *et*

*al.* (1991) examined the effects of adverse environmental conditions on spawning.

The timing of the various stages of gonad and gamete development that occur throughout the year has not been studied in detail for Atlantic cod. Burton *et al.* (1997) produced time series data for gamete development for cod from NAFO area 2J3KL but had to rely on a combination of wild and captive cod. The timing of oogenesis was examined for cod from the Flemish Cap in order to develop a method to identify females at the beginning of ripening (Zamarro *et al.* 1993).

Cod are batch spawners (Kjesbu & Kryvi 1989) and so release batches of hydrated oocytes at discrete intervals. The spawning pattern in this species has been described as determinate (Kjesbu *et al.* 1991) because a hiatus in size distribution exists between immature and vitellogenic oocytes prior to spawning. It has been shown for *Hippoglossoides platessoides* (Maddock & Burton 1998) and *Reinhardtius hippoglossoides* (Rideout *et al.* 1999), however, that *de novo* vitellogenesis can occur during spawning, making estimating fecundity very difficult, even though fecundity appears determinate before spawning. Since the spawning cod ovary is vitellogenically very active (Kjesbu *et al.* 1996), it may be possible for additional oocytes to be recruited into vitellogenesis during spawning. To date, however, there is no evidence for *de novo* vitellogenesis during spawning for this species (Kjesbu *et al.* 1990).

Little has been done to study the reproductive biology of cod in the inshore environment. It has historically been believed that most cod migrated between an offshore spawning area and an inshore feeding area (Lilly 1996). Evidence suggests that some

inshore spawning does occur but it is uncertain as to whether this indicates the existence of local stocks of inshore cod (Lilly 1996). With the realization that some cod remain inshore year round (Wroblewski *et al.* 1996; Bratley 1996), however, the existence of 'bay' stocks remains a possibility. Due to the collapse of offshore cod stocks the study of fish in the inshore environment may be more important than ever before and it has been suggested that inshore cod may make a larger contribution to recruitment than has previously been believed (Hutchings *et al.* 1993).

The purpose of the present study is to perform a baseline examination of the reproductive biology of female Atlantic cod from Placentia Bay, Newfoundland, including information on the spawning pattern, timing of reproductive development and seasonal changes in condition.

### 1.3 MATERIALS AND METHODS

#### Fish collection

A total of 226 Atlantic cod, *Gadus morhua*, were collected from Placentia Bay, Newfoundland using gill nets, hand lines and otter trawl. Sampling was restricted to the innermost part of the bay except in January, when fish were collected from Clattice Harbour (Fig. 1.1). Fish were collected in all months except March, September, November and February, as depicted in Table 1.1. Fork length, wet weight, gutted weight and gonad weight were recorded for all fish. Each fish was also assigned a visual, and later a histological maturity based on a scale produced by combining the ovarian descriptions of Morrison (1990), Walsh *et al.* (1986) and the unpublished work of the author (Table 1.2).

#### Condition indices

Condition factor ( $K_w$ ) and somatic condition factor ( $K_s$ ) were calculated by the formulae  $K_w = 100 W_w L^{-3}$  and  $K_s = 100 W_s L^{-3}$ , where  $W_w$  was whole weight in g,  $W_s$  was somatic or gutted weight in g and  $L$  was fork length in cm. Gonadosomatic index ( $I_G$ ) was calculated for all fish as  $I_G = 100 W_G W_w^{-1}$ , where  $W_G$  was gonad weight.

#### Histology

To determine a histological maturity stage, a small section of gonad from each fish was placed in Bouin's fixative (at the time of capture) for later examination in the

laboratory. Tissue samples were usually taken from the posterior part of one of the gonads but not collected consistently from the right or the left ovary since oocyte distribution has been determined to be consistent throughout and between both ovaries for this species (Holdway & Beamish 1985).

Upon removal from the Bouin's fixative, gonad samples were transferred to 50% ethanol for 30 minutes, then placed in 70% ethanol. Samples were then run through an ethanol dehydration series, cleared in xylene and embedded in Paraplast Plus®. Sections were cut at 7 µm on a rotary microtome and floated on distilled water on slides that had been smeared with Mayer's glycerine-albumin. The slides were then dried on a slide warmer at 37 °C. All sections were stained with Ehrlich's haematoxylin and eosin Y and then mounted in Histoclad®.

To elucidate the type of spawning for this species, oocyte frequencies were calculated for fish from various times of the year. Histograms of oocytes size frequencies were constructed for three prespawning cod from May, three spawning fish from July and three spent fish from August. A single gonad section was selected for each fish and three fields of view were randomly chosen. Only oocytes that were cut through the nucleus were included in the oocyte frequencies since this has been determined to be representative of the actual oocyte size (Foucher & Beamish 1980). The diameter of the oocytes was measured using an eyepiece micrometer and each recorded as either immature, circumnuclear ring stage, cortical alveoli stage, or vitellogenic. The percentage of oocytes in each 10 µm size class (i.e. 1-10, 11-20, etc.) as well as each stage of development was

calculated by dividing the combined number of oocytes of that size and stage from the three fields of view by the total number of oocytes measured.

## 1.4 RESULTS

### Oocyte development

Cod ovaries in all stages of maturity contain nests of oogonia, 5-10  $\mu\text{m}$  in diameter, which proliferate by mitosis (Fig. 1.2a), and form the basis for the production of all future oocytes. Oocyte growth proceeds through the chromatin nucleolar stage (12-20  $\mu\text{m}$ ) (Fig. 1.2b) and into the perinucleolar stage of development. Several sizes of perinucleolar stage oocytes are always present at the same time and are all characterized by numerous peripheral nucleoli (Fig. 1.2c). Early perinucleolar oocytes have a crescent-shaped basophilic area next to the nucleus. This basophilic area forms a horseshoe shape and eventually a complete ring encircling the nucleus, when the oocyte reaches 60-140  $\mu\text{m}$  in diameter. The circumnuclear ring migrates to the periphery of the oocyte and breaks down when the oocyte is 145-165  $\mu\text{m}$  wide (Fig 1.2d).

Cortical alveoli appear as clear vesicles in the periphery of the oocyte as the circumnuclear ring disintegrates. These structures are first visible in oocytes about 150  $\mu\text{m}$  wide and accumulate until the oocyte diameter is at least 250  $\mu\text{m}$  (Fig 1.3a). The cortical alveoli may continue to accumulate and grow as the oocyte grows but they are masked as eosinophilic yolk appears in oocytes between 180-250  $\mu\text{m}$  in diameter. Yolk continues to accumulate until the oocyte reaches 720-780  $\mu\text{m}$  (Fig 1.3b). The final stage of oocyte development before ovulation is hydration. Hyaline oocytes could not be measured in histological preparations since they took on an irregular shape (Fig. 1.3c).

### **Timing of ovarian development**

Spawning of female cod occurred between April and August (Figure 1.4). The percentage of fish with vitellogenic oocytes but which had not spawned yet (no post-ovulatory follicles, see below) decreased between April and June and no fish in this condition were found in July (i.e. fish with vitellogenic oocytes had spawned). By July, however, a small portion of fish had ovaries containing oocytes with cortical alveoli. The percentage of fish in this condition increased through to December and then dropped drastically. The first signs of vitellogenesis were in August and increased dramatically up to January. Females in the spent P condition (see Table 1.2) were collected between April and October but were not found in the December sample. A small portion of spent females was then recorded again in January.

The reproductive status of the population is reflected in the change in average  $I_G$  (Fig. 1.5). The ovaries were largest between April and August while fish were spawning and average  $I_G$  was lowest in the months following spawning.

### **Post-ovulatory follicle structure**

The ovulation of an oocyte leaves an empty follicle, known as a post-ovulatory follicle. Post-ovulatory follicles were identified as one of three basic types. These stages (A-C) are based on the structural changes of the theca and granulosa and represent sequential stages in the degeneration of the follicle. Type A follicles are large but highly contracted and were present in spawning and spent females in July and August. Type B



follicles were usually found along with those of Type A (Fig. 1.6a). These empty follicles exhibited signs of degeneration of the granulosa layer, which caused them to lose their tightly contracted nature. Empty follicles of this type were detected in small numbers in April and with high frequency in July and August. Type C post-ovulatory follicles are usually small with a thick layer of eosinophilic tissue (remains of theca?) surrounding several small aggregations of basophilic material (remains of granulosa?) (Fig. 1.6b). These empty follicles were detected in spent fish (with no signs of maturing for the next season) from August and October, fish undergoing the process of vacuolation in August, September and October and fish in the early (October and December) and late (April and May) stages of vitellogenesis (Fig. 1.6c). A fourth structure, identified in fish from all months, may represent an even further advanced state of degeneration of the follicle (Fig. 1.6d). It is uncertain, however, if such highly degenerated structures can successfully be distinguished from late stages of atresia.

#### **Ovarian development & spawning pattern**

All ovaries examined contained oocytes in the stages up to and including the circumnuclear ring stage of development. In maturing ovaries a hiatus in size distribution was seen between 'resting' perinucleolar oocytes and the growing vitellogenic oocytes (Fig 1.7). Ovaries that had begun spawning, as indicated by the presence of hyaline oocytes or new post-ovulatory follicles, still contained a pool of late vitellogenic oocytes that would have been spawned later and still displayed the hiatus in oocyte size (Fig 1.8).

Spent ovaries contained no vitellogenic oocytes (except sometimes 2-3 resorbing oocytes) and showed only a single mode of oocyte size in the frequency histogram (Fig 1.9).

All ovaries appeared to have fecundity determined before spawning except for two females collected in August. These fish were spawning and had a population of late vitellogenic oocytes still to be ovulated but also had a tiny mode of smaller vitellogenic oocytes (Fig 1.10, 1.11). Both fish were in good condition with  $K_w$  of 0.992 and 1.181 and  $K_s$  of 0.820 and 0.880.

### **Atresia**

Two main types of atretic follicles were observed in cod ovaries throughout the year: vitellogenic and previtellogenic. In both cases, oocyte degeneration occurred via the phagocytotic activity of hypertrophied follicle cells (Fig 1.12a, b). Atretic vitellogenic oocytes were observed in 64 (46.4 %) of the maturing and spawning ovaries (i.e. those with vitellogenic oocytes present). Previtellogenic atretic follicles, including those that reached the cortical alveoli stage, were observed in 27 (31.0 %) of the spent ovaries and those undergoing vacuolation. No previtellogenic atretic follicles were observed in ovaries with vitellogenic oocytes.

Mature degenerating oocytes were often encountered in small numbers after spawning (Fig 1.12c). Two females collected in July, however, appeared to be resorbing all or most of the vitellogenic oocytes that had developed the previous year (Fig 1.12d). No post-ovulatory follicles were present in these ovaries.

### **Cycle in condition indices**

The seasonal changes in average condition factor ( $K_w$ ) and somatic condition factor ( $K_s$ ) are depicted in Figure 1.13. There was a continuous build-up in  $K_s$  from April to October and a subsequent decrease to January. Average  $K_w$  increased between April and August and then decreased to December. It was elevated again slightly in January.

### **Accuracy of visual maturity scales**

Comparison of visual and histological maturity assessments revealed two problem areas in the use of visual maturity scales. First of all, two fish in April and four in August contained ovaries with newly formed (Type A and B) post-ovulatory follicles, indicating that they had commenced spawning but were visually assessed as still ripening (i.e. not spawning yet) because there were no clear (hyaline) oocytes present. Second, thirty-three ovaries collected between October and January had oocytes in the stages of cortical alveoli and early yolk formation but were visually assessed as being in the spent condition.

## DISCUSSION

Few studies have concentrated on the reproductive biology of inshore cod. Sample sizes are small in the present study but are sufficient to give some details of gonad growth and the timing of the reproductive cycle for Atlantic cod in Placentia Bay. Results confirm that cod spawn inshore during the spring and summer, which may indicate the existence of a coastal population of cod in this area ( Hutchings *et al.* 1993).

Oogenesis follows the same pattern for Atlantic cod as for many other fish (for reviews see Wallace & Selman 1981; Tyler & Sumpter 1996) with oogonia forming the basis for all oocytes to be produced. All females throughout the year contain oogonia and perinucleolar oocytes of various sizes, including those at the circumnuclear ring stage. Morrison (1990) determined the circumnuclear ring to consist of primarily mitochondria. The appearance of the circumnuclear ring, has been speculated to mark the oocytes that will mature and spawn next year (Holdway & Beamish 1985, Kjesbu & Kryvi 1989, Morrison 1990). More precisely, Shirokova (1977) suggested that it is the appearance of oocytes with circumnuclear rings beginning to degenerate that indicates a fish will mature. The circumnuclear ring was first described by Woodhead and Woodhead (1965) who claimed that all oocytes had to pass through the circumnuclear ring stage in order to mature but did not suggest that all oocytes at this stage would mature next year. All females collected in the present study contained circumnuclear ring stage oocytes and fish as small as 29 cm have been found to contain oocytes at this stage (Rideout, unpublished data). Fish this small were not likely to spawn in the next year, suggesting that oocytes at

this stage of development can last for longer than a year and that the appearance of these oocytes does not indicate that a fish will spawn in the next year. Cortical alveoli appear as the circumnuclear ring breaks down and are perhaps the best indicator that an oocyte will mature (Zamarro *et al.* 1993). Since cortical alveoli always appeared as the circumnuclear ring broke down, Shirokova's (1977) assumption that the breakdown of the ring indicates the onset of trophoplasmic growth (cortical alveoli accumulation and exogenous vitellogenesis) and the subsequent maturation of the oocyte may be valid. Exogenous vitellogenesis, the storage of yolk formed from hepatically derived vitellogenin, follows the appearance of cortical alveoli, and is followed by nuclear migration, disintegration of the nuclear membrane, hydration and ovulation.

Cod spawning in Placentia Bay took place over at least five months in 1998, which is not unusual for some stocks of Atlantic cod (Brander & Hurley 1992; Miller *et al.* 1995). The first signs of spawning were in April and the first ovaries with oocytes beginning to develop for the 1999 spawning season were collected in July. Individual cod spawn for up to 70 days (Kjesbu 1989), suggesting that there is at least a 1-2 month delay between spawning and recruitment of oocytes for the next year. Vitellogenic oocytes appeared for the first time in ovaries in August, suggesting that yolk accumulation begins a month or less from the first signs of cortical alveoli. All females were recruiting oocytes for the 1999 spawning season by December. Data indicate that vitellogenesis lasts for seven months or more.

In January a small percentage of females were observed to still be in the spent

condition (with Type C POFs) which may indicate that some fall spawning occurred in the bay. The 1-2 month time gap between spawning and recruitment indicates that these fish would have spawned somewhere around November. No other evidence was collected to indicate the presence of fall spawning fish in Placentia Bay, however, the January sample was the only sample from Clattice Harbour. All other samples were collected from the innermost part of the bay, where fall spawners may be rare. Fall spawners were found on the Scotian Shelf (McKenzie 1940) and there are some reports of small percentages of fall spawning cod in Placentia Bay (G. Rose, pers. comm.). An alternative explanation could be that these were spring spawners that were not going to take part in next year's spawning.

The presence of post-ovulatory follicles (POFs) are often used to indicate a recent spawning episode since they have been determined to last for only a few days in some warm water species (Hunter & Goldberg 1980). However, POFs have been estimated to last for seven to eight months in cod from the Flemish Cap area (Zamarro *et al.* 1993). In the present study, cod from Placentia Bay were estimated to have post-ovulatory follicles that persisted for up to nine months, since spawning ended in August and old POFs could still be identified in prespawning fish from May. Type A and B post-ovulatory follicles were determined to exist only during or immediately following spawning so these types of follicles could be used to indicate recent spawning activity. Empty follicles are of Type C 1-2 months after spawning and last for several months. It is common to see POFs in this stage of degeneration in fish with cortical alveoli stage oocytes and early vitellogenic

oocytes and can even be seen sometimes in ovaries with late vitellogenic oocytes. It is uncertain if follicles degenerated past the stage of Type C can be positively identified since old POFs may be difficult to distinguish from the late stages of reabsorption of oocytes.

It is well established that cod are batch (or serial) spawners (Kjesbu & Kryvi 1989) and this was evidenced in the present study by the observation that spawning fish had ovaries that contained a 'reserve' of late vitellogenic oocytes yet to be spawned but also had newly formed post-ovulatory follicles and/or hyaline oocytes indicating that some oocytes had already been ovulated. Batch spawning increases fecundity by overcoming the physical limitations of a small body cavity which limits gonad growth and increases the chance that at least some of the oocytes, and subsequent larvae, will coincide with favourable environmental conditions (McEvoy & McEvoy 1992). Kjesbu (1989) reported that cod spawn 10-15 batches of oocytes over 35-70 days.

The spawning pattern for Atlantic cod was reported by Kjesbu *et al.* (1991) as determinate because a gap in size distribution existed between previtellogenic and vitellogenic oocytes prior to spawning. Kjesbu *et al.* (1990) created size frequency distributions for spawning cod and saw no evidence for *de novo* vitellogenesis. In the present study, all ripening ovaries exhibited a hiatus in size between previtellogenic and vitellogenic oocytes. This was also the case for most spawning ovaries, but two spawning females collected in August had ovaries which contained numerous late vitellogenic oocytes, hyaline oocytes and newly formed post-ovulatory follicles, but also had a small mode of early vitellogenic oocytes. It is assumed that these smaller vitellogenic oocytes

indicate the ability of this species to recruit oocytes into vitellogenesis during spawning. Kjesbu *et al.* (1996) reported that the spawning cod ovary is still very vitellogenically active in that some oocytes are still accumulating yolk as others are being spawned. It is conceivable, then, that some oocytes may begin vitellogenesis at this time. The lack of early vitellogenic oocytes in spent fish suggests that these new oocytes go through vitellogenesis at an increased rate and are spawned towards the end of the present spawning season.

The recruitment of oocytes into vitellogenesis during spawning suggests an indeterminate form of spawning, but only for a small percentage of individuals in the population. A similar situation was reported by Horwood (1990) for *Pleuronectes platessa*, whereby some individuals were capable of pushing oocytes through vitellogenesis, which he referred to as 'fine tuning of fecundity', whereas others showed typical determinate spawning whereby fecundity was determined long before spawning. A similar form of indeterminate spawning has been suggested for some *Hippoglossoides platessoides* (Maddock & Burton 1998) and *Reinhardtius hippoglossoides* (Rideout *et al.* 1999), both of which appear to have determinate fecundity when examined before spawning. The occurrence of this form of indeterminate spawning suggests that fecundity may be being underestimated for some Atlantic cod but since evidence of this is rare, it is uncertain if it would be necessary to consider this in future fecundity studies.

The ability to recruit oocytes into vitellogenesis can act as a means of boosting fecundity when energy reserves are sufficient to do so. In contrast, the process of



follicular atresia can act as a mechanism to downgrade fecundity (Kjesbu *et al.* 1991) although Woodhead and Woodhead (1965) believed that no more than 2% of total fecundity was lost to oocyte degeneration. In the present study, the presence of atretic previtellogenic and vitellogenic oocytes was noted in ovaries throughout the year. Atresia always appeared to affect the most advanced oocytes in the ovary, with previtellogenic atresia in ovaries recovering from spawning and vitellogenic atresia in ripening and spawning ovaries. This is in contrast to the findings of Kjesbu *et al.* (1991) who found that atresia did not appear in the prespawning ovary. Atresia in this species has been shown to be inversely related to food availability (Kjesbu *et al.* 1991) so that increased atresia may be a means of using the energy stored in developing oocytes to fuel body maintenance functions when other energy sources are insufficient to do so.

The two females sampled in July 1998 which had undergone mass atresia of oocytes did not appear to have spawned. Skipped spawning in other species, such as *Pleuronectes americanus*, has been related to poor condition (Burton & Idler 1984). The individuals in question were in good condition at the time of capture (July) but may have been in poorer condition when gamete development was arrested. The ovaries of both females had oocytes that had begun to develop for the 1999 spawning season, indicating that the interruption in the spawning cycle was temporary.

The main protein depot for Atlantic cod is the body musculature (Love 1970) and somatic condition factor has been shown to be a good indicator of the amount of protein stored in the white muscle (Dutil *et al.* 1995). Condition factor based on whole weight is

not as good an indicator since it takes into account the weight of all organs including the gonad and liver instead of just the muscle mass (Dutil *et al.* 1995) as evidence by the maximum  $K_w$  in August. The lowest values for  $K_s$  were observed early in the spring and increased to a maximum in October. The increase in  $K_s$  suggests the replacement of protein stores back into the white muscle. Fish must have resumed feeding during or perhaps immediately following spawning. Feeding during or immediately following spawning is an important part of the energy storage cycle for some species (Paul *et al.* 1993; Burton 1994) and lack of sufficient food at this time can result in fish not developing gonads for the next spawning season (Burton 1994).

Average  $K_s$  decreased between October and January and likely continued to decrease through February indicating the mobilisation of protein from the muscle mass during late fall and throughout winter. Similar changes have been recorded for Pacific cod, *Gadus macrocephalus* (Smith *et al.* 1990) and for Atlantic cod from other areas and has been attributed to the use of muscle protein for the growth of the gonad (Damberg 1964; Love 1970). Eliassen & Vahl (1982), however, insist that changes in condition of Norwegian cod during the period of reduced feeding is due only to the mobilisation of energy stores for maintenance functions since immature fish showed similar changes in condition. No immature cod were analysed during this study and so the comparison of condition for immature and mature fish was not possible. Subsequently, it was not possible to determine if protein removed from the carcass was used simply as an energy source for body maintenance or if it also contributed to ovarian development during the

fall and winter. If the breakdown of white muscle energy stores can be used to fuel gonad growth than this may act as the energy source in some individuals to allow *de novo* recruitment of oocytes into vitellogenesis during spawning.

Two problems associated with the use of visual maturity categories became evident during this study. One problem is that oocytes can enter into vitellogenesis before they are visible to the naked eye and therefore females in the early stages of recrudescence are considered spent. To alleviate this problem, Zamarro *et al.* (1993) suggested the use of histology to examine gonads at the beginning of ripening. The second problem was the misidentification of some fish that had started spawning. Some female cod appear capable of spawning early batches of oocytes without leaving any hyaline oocytes behind. The absence of these clear oocytes resulted in the visual assessment that these fish have not started spawning. The presence of new POF's (Type A and B) suggests that one or more batches of oocytes had, in fact, already been spawned. Therefore histological techniques may also be useful to identify the start of spawning.

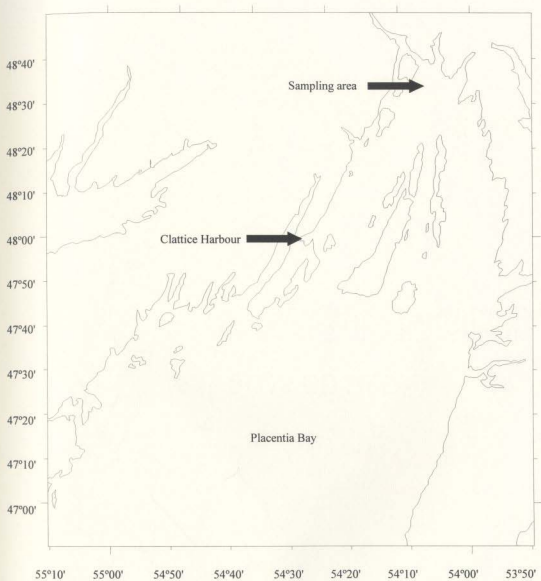


Figure 1.1. Map of Placentia Bay, Newfoundland showing sampling areas where female cod were collected in 1998-1999.

Table 1.1. Summary of number and size of female cod collected per sample.

Month	Sample Size	Fork Length (cm)	
		Range	Average $\pm$ Std. Dev.
April	21	53-91	66.0 $\pm$ 9.0
May	20	59-95	71.4 $\pm$ 11.1
June	25	60-94	71.4 $\pm$ 8.9
July	30	54-85	63.3 $\pm$ 7.6
August	32	52-90	66.0 $\pm$ 7.9
October	28	55-87	66.1 $\pm$ 6.4
December	29	56-85	66.7 $\pm$ 6.5
January	41	46-84	68.1 $\pm$ 8.9

Table 1.2. Maturity scale for female cod based on the combined descriptions of Morrison (1990), Walsh *et al.* (1986) and the unpublished observations of the authors.

Maturity Stage	General Description	
	Gross	Microscopic
<b><u>IMMATURE</u></b>	Ovary small. Translucent in small fish, but opaque, pinkish-orange in larger fish.	Ovary wall thin. Oogonia and a variety of sizes of chromatin nucleolar and perinucleolar stage oocytes. Largest oocytes with circumnuclear ring.
<b><u>MATURE</u></b>		
<b>Ripening</b>	Ovary becomes firm and eventually enlarges to occupy about half the body cavity. Becomes opaque and cream in colour with individual eggs visible with the naked eye.	<b>Cortical alveoli stage.</b> Oocytes with clear vacuoles in peripheral cytoplasm.  <b>Exogenous vitellogenesis.</b> Oocytes accumulate eosinophilic yolk, initially in peripheral cytoplasm and later filling entire oocyte.
<b>Ripe &amp; Spawning</b>	Ovary fills most of body cavity. Translucent as well as opaque eggs can be seen. Eggs easily released from vent at end of stage.	Oocytes hydrate in batches. Yolk appears as homogenous mass. Layer of cortical alveoli can be seen beneath egg membrane. Follicle and overlying ovigerous membrane ruptures, releasing egg into lumen. Post-ovulatory follicles in stage A and B of degeneration.
<b>Spent P</b>	Ovary shrunken, often soft and flabby. Red, sometimes with whitish cast.	Empty follicles (usually stage C) and a few atretic hydrated oocytes present. Perinucleolar stage oocytes also present.
<b>Spent L</b>	Visually similar to Spent P but observed at a time of year when ovary should be maturing. Ovary small, dull pink, sometimes with grayish cast. No eggs visible.	Mass atresia of oocytes at the beginning of vacuolation. These fish will not spawn in the upcoming spawning season.

Figure 1.2. Sections of Atlantic cod ovaries showing the stages of early oocyte growth.

(a) Nests of oogonia (**O**), mitotically active oogonia (**M**) (b) Immature gonad with chromatin nucleolar stage oocytes (**CN**) (c) Immature gonad with various sizes of perinucleolar stage oocytes (**P**), including some with a circumnuclear ring (**C**) (d) Immature ovary with oocytes at the late circumnuclear ring stage (**LCR**). (a) Scale bar = 50  $\mu\text{m}$ ; (b), (c) and (d) at same magnification; Scale bar = 100  $\mu\text{m}$ .

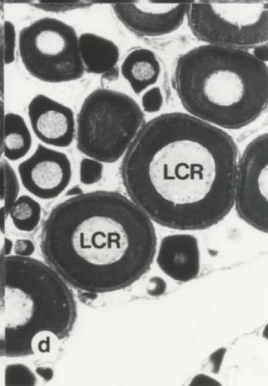
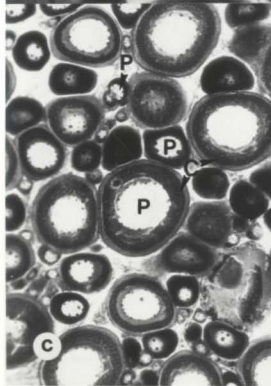
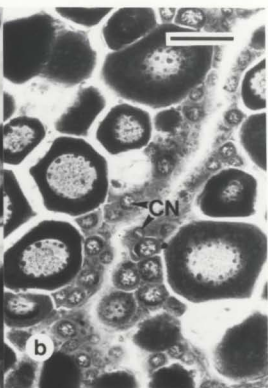
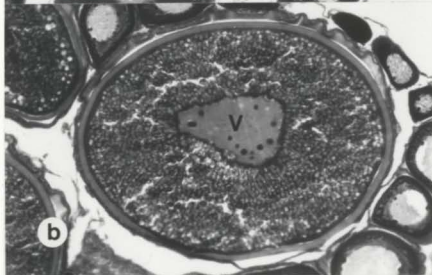
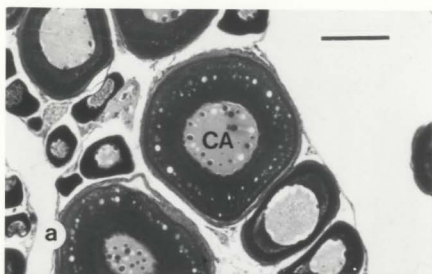




Figure 1.3. Sections of Atlantic cod ovaries showing the stages of late oocyte growth. (a) Cortical alveoli stage oocytes (**CA**) (b) Exogenous vitellogenic oocyte (**V**) (c) hydrated (hyaline) oocyte (**H**). (a) and (b) at same magnification; Scale bar = 100  $\mu\text{m}$  (c) Scale bar = 500  $\mu\text{m}$ .



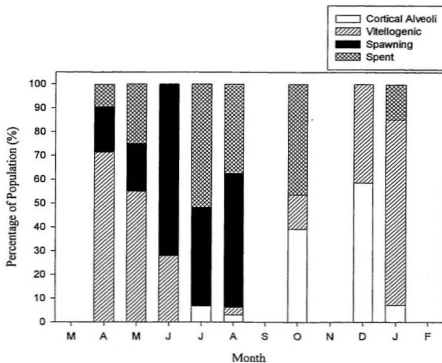


Figure 1.4. Allocation of maturities for monthly collections of female Atlantic cod from Placentia Bay in 1998-1999. Histological maturity stages are described in Table 1.2 and are based on the most advanced oocyte stage present.

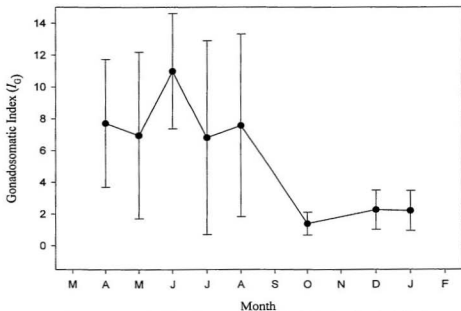
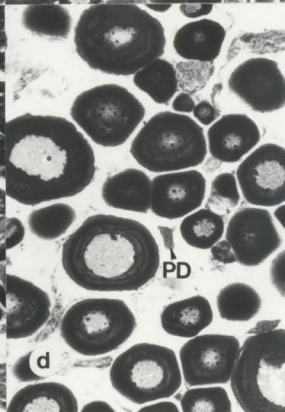
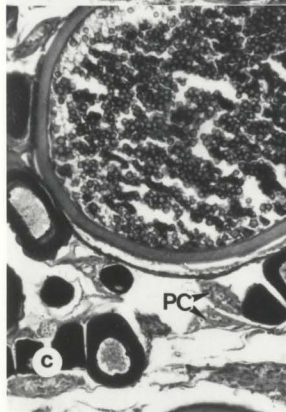
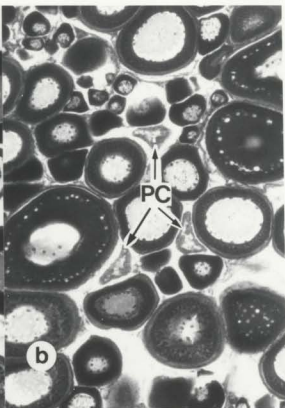
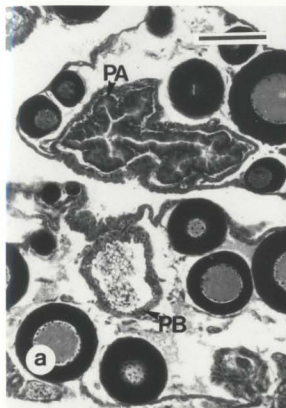


Figure 1.5. Cycle in average gonadosomatic index for female Atlantic cod from Placentia Bay in 1998-1999. Vertical bars = standard deviation.

Figure 1.6. Post-ovulatory follicle structure in Atlantic cod. (a) Recently spent ovary with Type A (**PA**) and Type B (**PB**) post-ovulatory follicles (b) Ovary recovered from spawning with Type C (**PC**) post-ovulatory follicles (c) Ripening ovary which still contains Type C post-ovulatory follicles from the previous year (d) Ovary with possible Type D (**PD**) post-ovulatory follicles. All figures at same magnification; Scale bar = 100  $\mu\text{m}$ .



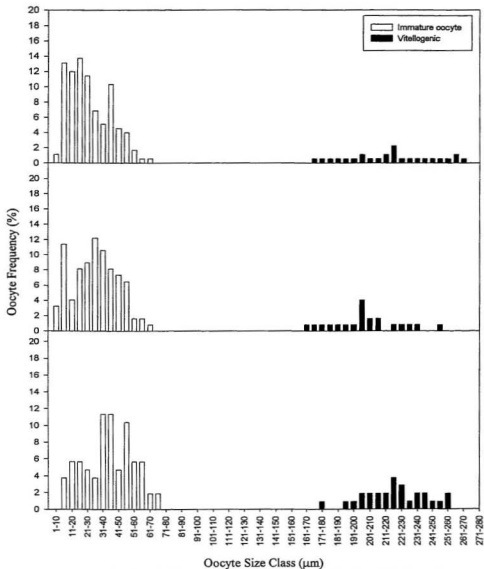


Figure 1.7. Oocyte frequency histograms for three ripening Atlantic cod from Placentia Bay in May (Above, length 92 cm,  $I_G$  7.7; Middle, length 61 cm,  $I_G$  9.4; Below, length 80 cm,  $I_G$  9.2).

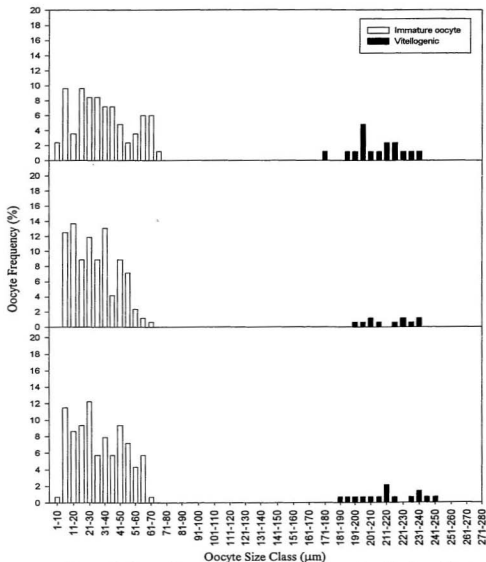


Figure 1.8. Oocyte frequency histograms for three spawning Atlantic cod from Placentia Bay in July (Above, length 55 cm,  $I_G$  8.3; Middle, length 63 cm,  $I_G$  10.0; Below, length 61 cm,  $I_G$  13.4).



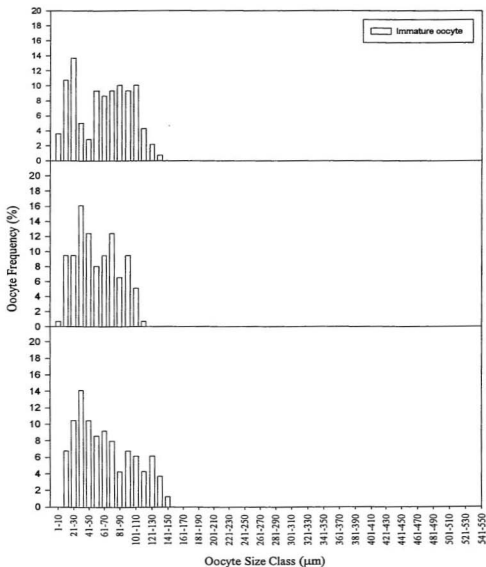
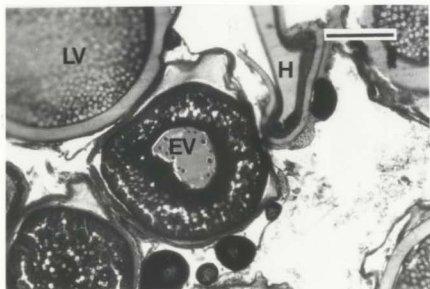


Figure 1.9. Oocyte frequency histograms for three recently spent Atlantic cod from Placentia Bay in August (Above, length 69 cm,  $I_G$  2.9; Middle, length 64 cm,  $I_G$  2.6; Below, length 74 cm,  $I_G$  2.7).

Figure 1.10. Section of the ovary of a spawning Atlantic cod from August (60 cm) showing recruitment of oocytes into vitellogenesis during spawning. Early vitellogenic (**EV**), late vitellogenic (**LV**) and hyaline oocyte (**H**). Scale bar = 100  $\mu$ m.



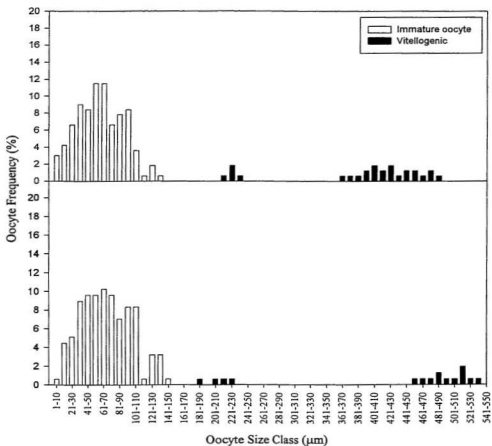
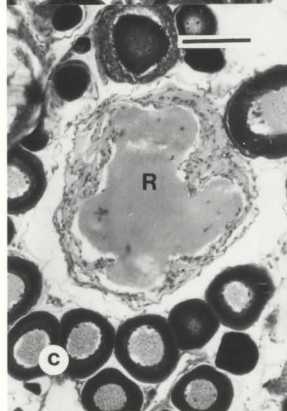
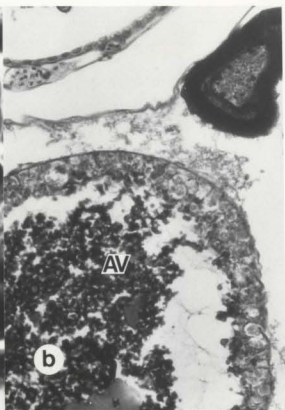
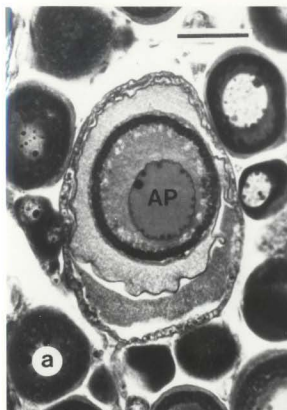


Figure 1.11. Oocyte frequency histograms for two spawning Atlantic cod from Placentia Bay in August showing signs of de novo vitellogenesis (Above, length 60 cm,  $I_G$  14.5; Below, length 64 cm,  $I_G$  6.4).

Figure 1.12. Sections of Atlantic cod ovaries containing atretic oocytes. (a) Ovary recovering from spawning with atretic previtellogenic oocyte (**AP**) (b) Ripening ovary with atretic vitellogenic oocyte (**AV**) (c) Spent ovary with mature resorbing oocyte (**R**) (d) Ovary collected in July exhibiting mass atresia of vitellogenic oocytes indicating spawning omission in 1998. Figures (a) and (b) at same magnification; Scale bar = 50  $\mu\text{m}$ . Figure (c); Scale bar = 100  $\mu\text{m}$ . Figure (d); Scale bar = 500  $\mu\text{m}$ .



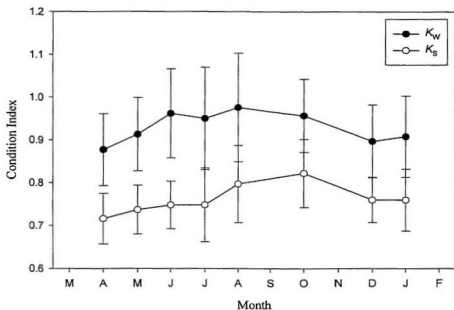


Figure 1.13. Comparison of condition factor indices using whole weight and gutted weight for female Atlantic cod from Placentia Bay in 1998-1999. Vertical bars = standard deviation.

## CHAPTER 2

### **The reproductive cycle of male Atlantic cod, *Gadus morhua* L., from Placentia Bay, Newfoundland.**

#### **2.1 ABSTRACT**

The timing of the reproductive cycle of male Atlantic cod, *Gadus morhua* L., from Placentia Bay, Newfoundland was determined from histological gonad samples collected throughout the year. Cycles in gonad, whole weight and muscle weight indices were also determined. Results were consistent with a spring spawning population, with males having testes filled with spermatozoa as early as January. There was a short resting period following spermiation, during which residual sperm was resorbed, followed by rapid development so that some sperm was again evident in the testes as early as November. At first, the presence of spermatozoa was accompanied by cells in all other stages of development, but by spermiation the testes were filled with virtually all spermatozoa. Gonad size was largest in April, smallest in October and increased steeply again in November. Energy for gonad growth came from food intake as well as liver lipid stores. Gonad development was, for the most part, finished prior to the winter reduction in feeding intensity.



## 2.2 INTRODUCTION

The process of spermatogenesis and, in general, male reproductive biology in teleost fishes has been studied to a much lesser extent than the reproductive development of females. Trippel and Morgan (1994) suggested that understanding the male's role in reproduction is important in developing sound management strategies for commercially exploited species. Monitoring gamete development in males, however, is somewhat problematic because the spermatogenic cells are small and therefore the different stages hard to identify (Morrison 1990) and formalin fixation makes identification even more difficult (Burton *et al.* 1997). Summaries of spermatogenesis in teleosts are given by Grier (1981) and Nagahama (1983), while Billard *et al.* (1982) discussed the endocrine control of spermatogenesis in fishes.

The literature available on spermatogenesis in teleosts is concentrated to a large extent on freshwater and anadromous fishes with studies conducted on brook stickleback, *Eucalia inconstans* (Ruby & McMillon 1975), perch, *Perca flavescens* (Turner 1919), brook trout, *Salvelinus fontinalis* (Henderson 1962), sockeye salmon, *Oncorhynchus nerka* (Weisel 1943) and rainbow trout *Oncorhynchus mykiss* (van Den Hurk *et al.* 1978). Most of the work on spermatogenesis in commercial marine species appears in the Russian literature. Sorokin and Grigoryev (1968) studied the annual spermatogenic cycle of Greenland halibut, *Reinhardtius hippoglossoides*, while the sexual cycle and spermatogenesis of Atlantic cod, *Gadus morhua*, was examined by Sorokin (1960) and Dobrusin (1970).

Henderson (1962) proposed that for spring spawning fish, there is a period of gonadal inactivity during the winter months and that this period may occur either before or following spermatogenesis. Some northwest Atlantic species such as winter flounder, *Pleuronectes americanus*, have been shown to complete spermatogenesis before entering a quiescent state which lasts throughout the winter (Burton & Idler 1987b; Moulton & Burton 1999). Burton and Idler (1984) reported that sperm in this species could be activated as early as January even though spawning doesn't occur until May-June. Atlantic cod have been reported to often have testes filled with fully developed sperm by February (Burton *et al.* 1997) perhaps indicating a winter quiescent period following spermiogenesis. Spermatogenesis, however, has not been monitored throughout the year for this species.

Seasonal cycles in energy content have been monitored for female cod and male and female cod combined but seldom has energy content or condition for male cod been studied separate from females. Karlson *et al.* (1995) performed a combined starvation and spawning study and found that males lost less somatic and liver weight than females and overall invested less in reproduction than females. Although the production of ova may require more energy than the production of sperm, mating behaviour of males may cause reproduction to be as energetically expensive as for females. This appears to be the case for *Mallotus villosus* whereby the cost of reproductive behaviour may be associated with low post-spawning survival rates for males (Burton & Flynn 1998). Spawning omission by male teleosts has been attributed to insufficient energy accumulation (Burton & Idler 1984;

Oganesyan 1993).

The intent of this study is to provide some baseline data on the yearly cycle of gametogenesis and the annual energy cycle for wild male Atlantic cod from Placentia Bay, Newfoundland which may be used to form a foundation for further, more detailed studies into the reproductive biology of this species.

## 2.3 MATERIALS AND METHODS

### Fish collection

Testes were removed from 272 Atlantic cod, *Gadus morhua*, collected by gillnet, bottom trawl and handline from Placentia Bay, Newfoundland between April, 1998 and January, 1999. The number and size of fish collected per month is shown in Table 2.1. Gonad weight was recorded along with a visually determined maturity stage based on the descriptions of Morrison (1990). Fork length, whole weight and gutted weight were recorded for each fish except that gutted weight was not recorded for the March sample. Gonadosomatic index ( $I_G$ ), condition factor ( $K_w$ ) and somatic (gutted) condition factor ( $K_s$ ) were calculated as:  $I_G = 100 W_G W_w^{-1}$ ,  $K_w = 100 W_w L^{-3}$  and  $K_s = 100 W_s L^{-3}$ , where  $W_w$  was whole weight in g,  $W_s$  was somatic or gutted weight in g,  $W_G$  was gonad weight in g and  $L$  was fork length in cm.

### Histology

A section was removed from the middle portion of each gonad and placed in Bouin's fixative for 48-72 hours. Tissue samples were dehydrated and embedded in Paraplast Plus® (as per Chapter 1) and sections were cut at 5  $\mu$ m using a rotary microtome. Ehrlich's haematoxylin and Eosin Y were used to stain all sections.

### Slide analysis

A second maturity stage was assigned to each testis based on histological analysis

and the following stages suggested by Grier (1981): (1) spermatogonial proliferation, (2) early recrudescence - spermatogonia and spermatocytes present, (3) mid recrudescence - all stages of sperm development present, (4) late recrudescence - lobules filled with sperm and number of developing sperm cysts is declining, (5) functional maturity - lobules filled with sperm, very little if any spermatogenesis is occurring, and (6) post spawned (spent).

Images of histological preparations were obtained for each testis using a Zeiss® microscope with Sony® videocamera and Bravado® image capturing board. Ten cells of every spermatogenic stage were measured in five fish using Mocha® video analysis software to give a total of 50 diameters for each cell type.

## 2.4 RESULTS

### Testis structure

The cod testes are paired organs in the caudal portion of the abdominal cavity and join posteriorly, opening to the genital pore via a sperm duct. The testes are suspended from the dorsal wall of the body cavity by a transparent peritoneal membrane. The structure of the testes was described by Morrison (1990). The part of the testes nearest the peritoneal membrane, the proximal testis, is an elongate tubular structure (consisting primarily of sperm duct) and from it arises the distal testis, a frill-shaped extension that becomes greatly enlarged as the testes develop.

### Cellular changes during spermatogenesis

The sequence of cellular changes that occurs during spermatogenesis is the same for Atlantic cod as for other species of teleosts. The cell size of the developing gametes decreased as they became more developed (i.e. closer to becoming spermatozoa) as is shown in Table 2.2. Spermatogonia were lightly staining cells with a large nucleus and single nucleolus. Primary spermatocytes, secondary spermatocytes and spermatids all had condensed chromatin causing them to be haematoxylinophilic. The most basophilic cell type was spermatozoa which could be identified by the presence of eosinophilic flagella.

### Pattern of spermatogenesis

Little consideration was made of immature males in the present study, since only

three were collected during sampling (one in June and two in January). These limited specimens showed that immature testes contain only spermatogonia. The conversion of spermatogonia into primary spermatocytes signified the beginning of ripening and appeared to occur almost simultaneously throughout the testis, whereby all developing cells proceeded to the primary spermatocyte stage before any cells proceeded through meiosis (Fig. 2.1a).

Cysts of primary spermatocytes began meiosis at different times and perhaps proceeded through meiosis at different rates so that spermatogenesis was asynchronous, often with spermatozoa, primary spermatocytes and stages in between all present in the testis at the same time (Fig. 2.1b). Secondary spermatocytes, however, were the least frequently observed stage of development in the testes examined. It was possible for primary spermatocytes, spermatids and spermatozoa to be present without seeing any secondary spermatocytes. Development was always synchronous amongst all the cells in an individual cyst.

Spermatozoa were initially arranged in such a way that the heads of all the cells derived from a single cyst were grouped together with the flagella oriented in the same direction. The order of the sperm arrangement eventually broke down and the sperm became loose in the testis. Areas of loose and connected sperm were usually present at the same time. In the latest stages of development the testes contained almost all loose sperm (Fig. 2.1c).

Spent testes had numerous empty ducts or small amounts of residual sperm

remaining in the ducts. In cod, the distal testis usually had recovered from spawning (and sometimes began to ripen again) while the proximal testis still contained large amounts of residual spermatozoa (Fig. 2.2a). The reabsorption of residual spermatozoa from the testis occurred via phagocytes, 3-12  $\mu\text{m}$  in diameter (Fig. 2.2b), and sometimes by the hypertrophied cells lining the sperm ducts (Fig. 2.2c). Recovery of testes from spawning was accomplished by the mitotic proliferation of reserve spermatogonia. Mitosis continued until the testis appeared immature-like, being filled with spermatogonia (Fig. 2.2d).

#### Timing of spermatogenesis

Male cod in May contained some amount of spermatozoa and in June, July and August most fish had testes that were completely filled with spermatozoa (Table 2.3). In October, the process of regenerating the line of germ cells within the testis had begun and by November, December and January the testes of most fish were again in the early stages of sperm formation. By January some testes were filled with sperm. The percentage of fish in each maturity stage throughout the year can be used to give a complete annual time course for spermatogenesis for Atlantic cod from Placentia Bay (Fig 2.3). Of note is the fact that fish with running milt were only collected in June and July, while testes filled or nearly filled with spermatozoa were collected from January through to August.



#### Annual Cycle in $I_G$ , $K_w$ & $K_s$

Average  $I_G$  values calculated per month (Fig. 2.4) showed a seasonal trend, decreasing from April to October and subsequently increasing from October to January. Cycles for  $K_w$  and  $K_s$  are shown in Figure 2.5. Average  $K_s$  increased between April and August, remained somewhat constant between August and November, and decreased between November and January.  $K_w$  showed a different pattern with two peaks, one in May and one in November.

#### Testis Wall

The thickness of the testis wall was not uniform throughout the entire organ (See Fig. 2.2a). Wall thickness was always greatest in the proximal testis. In the distal testis, wall thickness was greatest at the connection point to the proximal testis and decreased towards the outer tip of the lobes of the distal testis.

## 2.5 DISCUSSION

The reproductive strategy of male Atlantic cod appears to be to produce most or all of the sperm that will be needed during mating by early winter (November for Placentia Bay cod) and then to hold this sperm until the spring spawning period. This is consistent with Henderson's (1962) second category of reproductive development whereby spermatogenesis is completed before the winter months during which there is a period of gonadal quiescence. The same pattern of development is shown by *Pleuronectes americanus* (Burton & Idler 1984; Moulton & Burton 1999) which has spermatozoa in the testes six months prior to spawning. An alternative pattern of development utilized by *Salvelinus fontinalis* is to leave testes in an undeveloped state for most of the winter and then produce spermatozoa 1-2 months prior to spawning (Henderson 1962).

The production of spermatozoa before winter may be a means of increasing the chances of successfully overwintering. Atlantic cod undergo reduced feeding or complete fasting during winter months. During this period cod are dependent on stores of lipids in the liver and protein in the white muscle to fuel all energy requirements including gonad growth (Damberg 1964; Love 1970; Krivobok & Tokareva 1973; Eliassen & Vahl 1982; Kjesbu *et al.* 1991). If gonad growth is completed (i.e. mature gametes produced) while the fish is still feeding then there is likely minimal impact on the fish's energy reserves, increasing the chance of surviving until feeding begins again.

The time series created for spermatogenesis did not show any evidence of fall spawning, giving a complete reproductive cycle for spring spawning cod from Placentia

Bay. Spent males were first observed in July and those that had testes with proliferating spermatogonia were first observed in August, likely suggesting that there is a short (perhaps 4-6 weeks) 'resting period' following spermiation during which the reabsorption of residual spermatozoa takes place. There is a two month interval between the beginning of spermatogonial proliferation and the conversion of spermatogonia into primary spermatocytes, which were first seen in October. Just one month later the majority of testes sampled contained some spermatozoa, indicating that spermatogenesis occurred very rapidly. Spermatogenesis appears to be asynchronous during this rapid period of development with all cell stages sometimes being present. A similar pattern of asynchronous development was described for *P. platessa* (Barr 1963). The process of spermiogenesis continues to occur for at least a couple of months. In the initial stages there are only scattered areas of spermatozoa but eventually the testis becomes filled with spermatozoa.

The time series presented here is very similar to that produced by Burton *et al.* (1997) for Atlantic cod from NAFO area 2J3KL. Two main differences exist between the two time scales. First of all the time series of Burton *et al.* includes a reproductive category in their maturity scale in which the most advanced germ cells are spermatids. Testes of this category were not observed in the present study. Whenever spermatids were present, spermatozoa were also present, although sometimes in very small amounts. The second difference involves the length of time that testes were determined to contain predominately spermatozoa. This was observed from January to August in the present

study but only between February and May in Burton *et al.*'s study.

Although male cod with testes filled or nearly filled with spermatozoa were collected from January to August, running milt was only observed in June and July. It is unlikely that the release of milt only occurred in these two months, especially since females in spawning condition were collected from Placentia Bay between April and August (Chapter 1). The early production of spermatozoa ensures that males will be ready to spawn whenever females become ready. The release of milt may occur only when environmental conditions are right (i.e. ripe females present). This would be an adaptive strategy that would allow maximum fertilization. Although the spermatozoa of Atlantic cod remain viable in seawater for quite a long time (up to an hour) (Trippel 1994), the mating behaviour (synchronous release of male and female gametes) ensures the maximum encounter between sperm and oocytes and thus maximum fertilization.

Somatic condition factor is a good indicator of the energy reserves in the white muscle mass for Atlantic cod (Dutil *et al.* 1995). The increase in  $K_s$  between April and August, indicates that fish were feeding throughout this period and energy was being stored in the white muscle in the form of protein. The lack of a decrease in  $K_s$  between August and November, the period during which most spermatogenic activity occurred, indicates that gonad growth is completely fuelled by liver lipids and the food being consumed. Undoubtedly liver lipids contribute to gonad growth, however, liver weights were not available so it is uncertain what proportion of energy is supplied by liver and what proportion by food intake. It is known, however, that male gonad growth is less

energetically demanding than in female cod (Karlsen *et al.* 1995). After November,  $K_s$  decreased signifying a reduction in feeding intensity. Most gonad development was completed by this time so all energy was being used for basic metabolism.

Changes in  $K_w$  did not follow the same pattern as  $K_s$ . There were two peaks in  $K_w$ , one in May during spawning and the other in November. The peak in November is a result of the high  $K_s$  of the fish at this time coupled with the onset of spermiogenesis which causes a large increase in gonad size. Since  $K_w$  takes into account both the somatic weight of the fish as well as the weight of all the organs, including gonad, it is not as a good an indicator of somatic energy content for Atlantic cod (Dutil *et al.* 1995).

No evidence was collected during this study to suggest that male cod in Placentia Bay were undergoing spawning omission. Gonad development in the males of this species is not as energetically demanding as for females (Karlsen *et al.* 1995). This is supported by the observation that cod raised in captivity on low food rations still had ripening gonads in January even though condition factors were as low as 0.594 and white muscle water content was over 85% (Rideout, unpublished data). Non-reproductive males are reported, however, for both Atlantic cod (Oganesyan 1993) and *P. americanus* (Burton & Idler 1984). Burton *et al.* (1997) used testicular wall thickness to identify non-reproductive male cod but the present study suggests exercising caution when using this technique since wall thickness is not uniform throughout the testis. Using a standard sampling position on the testis is recommended. Perhaps the best position for comparative purposes would be on the distal testis wall, half-way between the point where the distal testis joins the

proximal testis and the tip of the lobe of the distal testis.

Table 2.1. Summary of number and size of male cod collected per sample.

Month	Sample Size	Fork Length (cm)	
		Range	Average $\pm$ Std. Dev.
March	26	39-108	60.8 $\pm$ 22.9
April	27	36-88	60.0 $\pm$ 14.1
May	18	59-95	71.4 $\pm$ 11.1
June	29*	60-94	71.4 $\pm$ 8.9
July	30	54-85	63.3 $\pm$ 7.6
August	24	52-90	66.0 $\pm$ 7.9
October	28	57-77	67.1 $\pm$ 6.3
November	28	57-70	64.9 $\pm$ 4.2
December	32	56-85	66.7 $\pm$ 6.5
January	30*	46-84	68.1 $\pm$ 8.9

\*One immature fish from June and two from January not included.

Table 2.2. Range of cell sizes for the different stages of germ cell development during spermatogenesis for Atlantic cod.

Cell stage	Cell diameter ( $\mu\text{m}$ )
Primary spermatogonia	7.5-8.0
Secondary spermatogonia	4.0-5.0
Primary spermatocytes	2.0-3.0
Secondary spermatocytes	1.2-1.5
Spermatids	0.9-1.0
Spermatozoa	0.8-1.0



Figure 2.1. Sections of Atlantic cod testes showing the sequential stages of spermatogenesis. (a) Testis full of primary spermatocytes (SC1) (b) Testis showing asynchronous development with stages from primary spermatocytes to spermatozoa (S) present (c) Testis filled with spermatozoa. All figures at same magnification; Scale bar = 50  $\mu$ m.



Figure 2.2. Sections of Atlantic cod testes recovering from spermiation. (a) Distal testis (**DT**) recovering while proximal testis (**PT**) still contains spermatozoa (b) Elimination of residual spermatozoa by phagocytes (**PH**) and by (c) the hypertrophied cells (**HC**) lining the sperm duct (d) Resting (**SG**) and mitotically (**M**) active spermatogonia. Figures (a), (b) and (c) at same magnification; Scale bar = 50  $\mu\text{m}$ . Figure (d); Scale bar = 50  $\mu\text{m}$ .

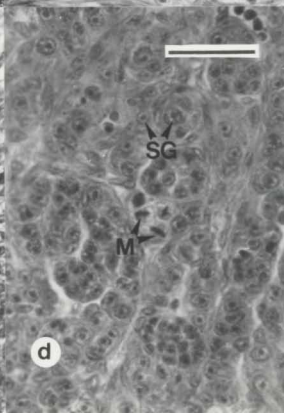
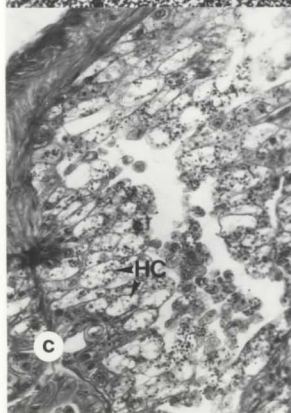
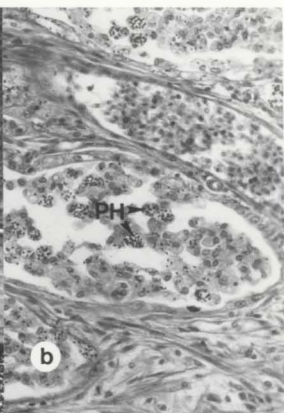
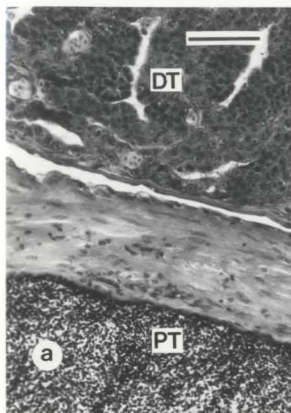


Table 2.3. Allocation of maturities for monthly collections of male cod from Placentia Bay in 1998-1999 (Numbers are percentages, maturity stages are those of Grier (1981)).

Month	Maturity					
	Spermatogonial proliferation	early recrudescence	mid recrudescence	late recrudescence	functional maturity	post spawned
March	-	-	7.7	23.1	69.2	-
April	-	-	-	63.6	36.4	-
May	-	-	33.0	33.0	33.0	-
June	-	-	-	13.8	86.2	-
July	-	-	-	6.7	66.7	26.6
August	16.6	-	-	-	66.7	16.7
October	64.3	28.5	-	-	-	7.2
November	14.3	14.3	71.4	-	-	-
December	6.3	12.5	81.3	-	-	-
January	-	16.7	53.3	13.3	16.7	-

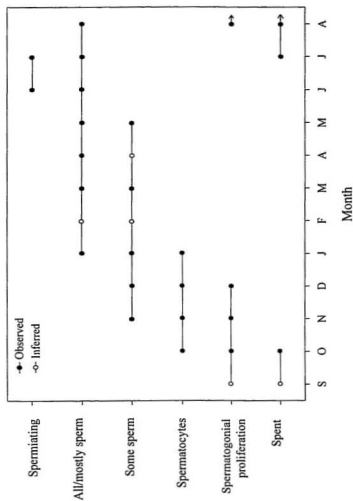


Figure 2.3. Time course of spermatogenesis for Atlantic cod from Placentia Bay, Newfoundland.

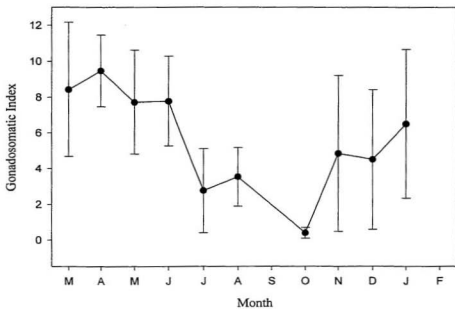


Figure 2.4. Cycle in average gonadosomatic index for male Atlantic cod from Placentia Bay in 1998-1999. Vertical bars = standard deviation.

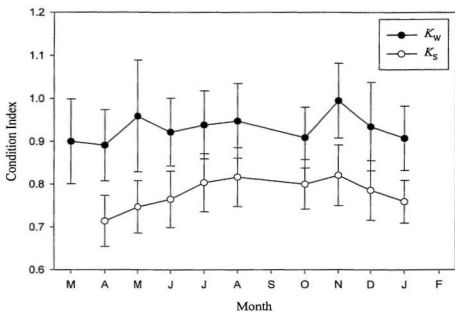


Figure 2.5. Comparison of condition factor indices using whole weight and gutted weight for male Atlantic cod from Placentia Bay in 1998-1999. Vertical bars = standard deviation.



## CHAPTER 3

### **The reproductive status of Atlantic cod, *Gadus morhua* L., from Smith Sound, Newfoundland: An investigation into the act of spawning omission by mature individuals.**

#### **3.1 ABSTRACT**

Atlantic cod, *Gadus morhua*, were collected from Smith Sound, Newfoundland in January 1999 during an acoustic survey of the area. Visual examination of both female (n=150) and male (n=126) gonads revealed fish that were immature, ripening for the upcoming spawning season, and fish that appeared to have spawned in the previous year but showed no signs of ripening for the upcoming spawning season. Histological examination of ovaries revealed that the majority of fish in the latter category were undergoing mass resorption of oocytes, most of which had reached the endogenous yolk stage. Hepatosomatic indices for these fish were significantly lower ( $p<0.05$ ) than ripening females and did not differ ( $p>0.05$ ) from that of immature individuals which may indicate that the interruption in the maturation cycle was due to insufficient nutrient storage required to allow normal ovarian maturation. Testes that were classified as delayed showed the presence of residual sperm indicating previous spawning but the remainder of the testis was filled with spermatogonia indicating that these fish had not started to ripen for the upcoming spawning season. These fish had hepatosomatic indices significantly higher ( $p<0.05$ ) than immature and ripening fish. It is uncertain if delayed males could still have matured for the spring spawning season. The current study indicates the need for

histological examination of gonads when assessing fish maturities.

### 3.2 INTRODUCTION

In the reproductive cycle of fishes there is no return to an immature state after the onset of maturation. The use of the term 'mature' indicates that a fish is spawning or has spawned at some time in the past (i.e. is not immature). Several studies report the existence of mature non-reproductive fish indicating that maturation need not imply annual spawning, with non-spawning mature fish having gonads that often macroscopically appear immature. Species for which spawning omission has been noted in the wild include winter flounder, *Pleuronectes americanus* (Burton & Idler 1984), American plaice, *Hippoglossoides platessoides* (Pitt 1966), Hoki, *Macruronus novaezelandiae* (Livingston *et al.* 1997), burbot, *Lota lota* (Pulliainen & Korhonen 1990), yellow-fin bream, *Acanthopagrus australis* (Pollock 1984), orange roughy, *Hoplostethus atlanticus* (Bell *et al.* 1992) and Greenland halibut, *Reinhardtius hippoglossoides* from the Barents Sea (Fedorov 1968, 1971) and the Northwest Atlantic (Walsh & Bowering 1981).

The failure of mature gonads to ripen has been attributed to adverse environmental conditions (Fedorov 1971) or to poor fish condition (Burton & Idler 1984; Walsh *et al.* 1986). Experiments have shown that fecundity decreases with reduced food rations (Scott 1962; Hester 1964; Bagenal 1969; Wootton 1973; Hislop *et al.* 1978; Kjesbu *et al.* 1991) and starvation or severe food restriction has produced post-mature non-reproductive *P. americanus* (Burton & Idler 1987a; Maddock & Burton 1994), haddock, *Melanogrammus aeglefinus* (Hislop *et al.* 1978) and *P. platessa* (Rijnsdorp 1990). Rijnsdorp (1990) noted, however, that this phenomenon of skipping a spawning season does not seem to occur in

wild North Sea plaice. Determining the frequency of skipped spawning would allow more accurate calculations of spawning biomass and be very useful for stock assessment purposes.

Spawning periodicity has not been thoroughly studied for Atlantic cod but annual spawning is often assumed. It is known that cod depend on protein and lipid reserves for gonad maturation (Damberg 1964; Krivobok & Tokareva 1973; Kjesbu *et al.* 1991) and restricted feeding regimes given to captive cod result in reduced fecundity (Kjesbu *et al.* 1991). Very few cod have been induced to omit spawning by food restrictions (Karlsen *et al.* 1995, Burton *et al.* 1997). Greer-Walker (1971) was able to produce non-reproductive cod, but only after complete starvation for 130 days. Despite this, there are some reports of non-reproductive adult cod from wild populations. Oganessian (1993) reported 22-26% of large female cod and 13% of males in various parts of the Barents Sea that were undergoing spawning omission. Burton *et al.* (1997) reported a very small number of non-reproductive adults from NAFO area 2J3KL. Walsh *et al.* (1986) reported that during the 1978-85 period on average 1/3 of mature females from the Flemish Cap did not spawn.

During April 1995 a school of adult cod with an estimated biomass of 17,000 tons was located in Smith Sound, Trinity Bay (Rose 1996). These fish reside in the bay year-round (Wroblewski *et al.* 1996; Bratney 1996), providing an easily accessible population to study various aspects of maturation and spawning. Spawning aggregations have been observed in the bay in April (Bratney 1996) and July (Wroblewski *et al.* 1996) indicating a spring spawning season. There are reports of large cod (>60 cm) in this area with

apparently immature gonads and it has been suggested that these fish may represent mature females that would not have spawned that year (Bratley 1996).

With large schools of cod being discovered inshore and the suggestion that these fish may make a larger contribution to cod recruitment than has previously been believed (Hutchings *et al.* 1993) it has become exceedingly important to study the reproductive biology of inshore cod including spawning periodicity. The purpose of this study is to examine the reproductive status of Atlantic cod from Smith Sound, Trinity Bay and determine if there is evidence of spawning omission.

### **3.3 MATERIALS AND METHODS**

#### **Fish**

A total of 150 female and 126 male cod were collected from Smith Sound, Trinity Bay, Newfoundland (Fig 3.1) in January of 1999 during an acoustic survey of the area. Data collected on all fish included fork length, whole weight and gonad weight and each fish was assigned a maturity based on visual inspection of the gonad. Gutted weight and liver weight were also recorded for some individuals. For 106 of the female and all 126 of the male fish a small piece of posterior gonad was excised and placed in Bouin's fixative for subsequent histological analysis. Thirty nine females were easily identifiable as ripening for the next spawning season by the presence of opaque oocytes. These fish were called ripening based on visual inspection alone since it has been shown that visual identification of this maturity stage is 100% accurate (Walsh *et al.* 1986). The other five ovaries were frozen immediately after removal from the fish and returned to the lab where photographs were taken. Sections of the gonads were then removed and placed in Bouin's fixative as per the fresh gonad samples.

#### **Tissue preparation and histology**

Gonad samples were left in Bouin's fixative for 48-72 hours, then placed in 50% ethanol for 30 minutes and finally transferred to 70% ethanol. Embedding, sectioning and staining were done as per chapters 1 and 2. Ovarian maturities based on histology were determined using a maturity scale that combined the histological descriptions of Morrison

(1990), Walsh *et al.* (1986) and the unpublished observations of the authors (Ch. 1 Pg. 26). For one ovarian section per slide the thickness of the ovarian wall was measured in at least four places using a calibrated eyepiece micrometer. Male maturities were staged according to the descriptions of Morrison (1990) (Ch. 2).

### **Statistics**

For all fish, condition factor ( $K_w$ ) and gonadosomatic index ( $I_G$ ) were calculated as  $K_w = 100 W_w L^{-3}$  and  $I_G = 100 W_G W_w^{-1}$  where  $W_w$  was whole weight and  $W_G$  was gonad weight in g, and  $L$  was fork length in cm. For those fish which liver and gutted weights were collected for, somatic (gutted) condition factor ( $K_s$ ) and hepatosomatic index ( $I_H$ ) were calculated as  $K_s = 100 W_s L^{-3}$ , and  $I_H = 100 W_H W_w^{-1}$ , where  $W_s$  was somatic or gutted weight and  $W_H$  was liver weight in g.

Regression analyses were performed ( $\alpha=0.05$ ) to determine if fish length affected  $K_w$ ,  $K_s$ ,  $I_G$ ,  $I_H$  and ovarian wall thickness. Comparison of  $K_w$ ,  $K_s$ ,  $I_G$ , and  $I_H$  between maturity stages was done by ANCOVA (covariate=length,  $\alpha=0.05$ ) as was the comparison of ovarian wall thickness.

Maturity ogives were constructed for both males and females and the percentage of females in each size group that would have spawned in the 1999 spawning season was determined.

### 3.4 RESULTS

All cod ovaries collected during this study were visually assessed as being immature, ripening or spent L. No spawning ovaries were observed. The accuracy of these gross maturities is presented in Table 3.1. Problems of accuracy were associated with small ovaries in which no oocytes were visually identifiable (i.e. immature and spent L). Spent L ovaries could histologically be divided into three categories: (1) immature, (2) those that had begun to ripen but were not far enough along to distinguish oocytes visually, and (3) those that were resorbing all oocytes that had started to ripen. The ovaries that exhibited mass atresia could be divided into those that showed signs of spawning previously (POFs), which were referred to as non-reproductive, and those that showed no signs of ever spawning, referred to as adolescent (Fig 3.2). Females visually assessed to be immature also contained some adolescent and non-reproductive individuals. Ovaries undergoing mass atresia of ripening oocytes appeared similar to recently spent ovaries with a dark pinkish-red colour, sometimes with a grey cast, but in rare cases appeared pinkish-orange like an immature ovary (Fig 3.3).

The effect of erroneously assigned maturities on the construction of maturity ogives for female cod is shown in Figure 3.4. The visual maturity ogive presents a steeper slope than the histological ogive while the graph of percent spawners shows that not all mature females would have spawned in 1999.

Table 3.2 summarizes the average values for various condition indices and other measurements for the four female maturities. Regression analysis revealed that  $K_w$ ,  $K_g$  and



ovarian wall thickness were significantly related to body size for immature fish but  $I_G$  and  $I_H$  were not related to body size (Table 3.3). Ovarian wall thickness,  $K_W$ ,  $K_S$ ,  $I_G$  and  $I_H$  were independent of body size for adolescent, ripening and non-reproductive cod.

There was no significant difference in  $K_W$  or  $K_S$  for immature, adolescent and non-reproductive females (Table 3.4). Ripening fish had significantly higher  $K_W$  than adolescent and non-reproductive fish but  $K_S$  was not different. Gonadosomatic indices differed significantly between all four maturities and increased in the following order: immature, adolescent, non-reproductive and ripening. Immature, adolescent and non-reproductive females did not have significantly different  $I_G$  but all were significantly lower than for ripening females.

Adolescent fish had significantly thicker ovarian walls than immature females and ripening fish had significantly thicker walls than adolescents. The ovarian walls of ripening and non-reproductive ovaries showed no significant difference in thickness and both were significantly thicker than immature and adolescent ovaries.

Male fish were visually identified as immature, ripening or Spent L. There were no signs of running milt. Comparison with histologically assessed maturities showed visual maturities to be fairly accurate for males (Table 3.5 and Figure 3.5). The main problem was with males that were assessed as Spent L. Over half of these fish had begun ripening. Histologically, male fish were recorded as being either immature, ripening (sperm absent), ripening (starting spermiogenesis), ripening (late spermiogenesis) or delayed. Testes categorized as delayed contained small to medium amounts of residual sperm with

spermatogonia constituting the rest of the gonad. Comparison of  $K_w$ ,  $K_s$ ,  $I_G$  and  $I_H$  showed no significant difference for ripening males in the early and late stages of spermiogenesis. Subsequently, the data for these two stages was pooled as ripening (sperm present) leaving a total of four maturity stages (Fig 3.6).

The average condition indices for the male maturities are presented in Table 3.6. Both  $K_w$  and  $K_s$  were found to be independent of fish size for all maturities (Table 3.7). There was a significant relationship between  $I_G$  and length for immature testes and testes that were ripening (sperm absent) but not the other two maturities. The liver index ( $I_H$ ) was independent of fish size for all maturities except delayed, where a significant relationship existed.

There was no significant difference in  $K_w$  for ripening (sperm absent), immature and delayed males but  $K_w$  for ripening (sperm present) males was significantly greater than these three (Table 3.8). No significant difference existed in the  $K_s$  of any of the four maturities. Ripening (sperm present) and ripening (sperm absent) males had significantly higher  $I_G$  than immature and delayed fish. The  $I_G$  of ripening (sperm present) males was significantly higher than ripening (sperm absent) males. Delayed males had significantly higher  $I_H$  than the other maturities, which did not differ significantly from each other.

### 3.5 DISCUSSION

A fairly large proportion of the female cod collected in this study had ovaries that were small in size and appeared superficially to be spent. Most of these large cod with low gonadosomatic indices had ovaries that were undergoing mass atresia of all oocytes that had begun to develop for the spring spawning season. Kjesbu *et al.* (1991) reported that for spring-spawning Norwegian cod, vitellogenesis commenced between September and November and Burton *et al.* (1997) reported that changes signifying that a female will be reproductive are detectable approximately seven months or more before spawning for Northwest Atlantic cod. This indicates that female cod resorbing all developing oocytes would not have been able to mature other oocytes to be spawned in the spring spawning period and thus those fish that had spawned in a previous year would have skipped this year's spawning and those females that had never spawned would have failed to mature this year.

Mass atresia of oocytes was reported for a small number of Atlantic cod from the Barents Sea which had gonads that visually appeared to be immature (Woodhead and Woodhead 1965). Walsh *et al.* (1986) reported mass atresia of oocytes in the ovaries of Flemish Cap cod and estimated that only 1/3 of the spawning biomass in this area actually spawned in any given year between 1978-85. The active resorption of all developing oocytes was also reported for Greenland halibut, *Reinhardtius hippoglossoides*, from the Barents Sea (Fedorov 1968; 1971).

Small percentages of atretic oocytes appear to be common in the ovaries of teleost

fishes. Mass atresia, however, is not a common event and likely indicates that an animal has had severe stress placed on it by the abiotic and/or biotic factors of its environment. Fedorov (1968, 1971) attributed the mass atresia of oocytes in Greenland halibut to unfavourable environmental conditions, particularly cold water currents. Winter flounder, *Pleuronectes americanus*, which were considered to be skipping a spawning season, had lower condition than those that were successfully developing toward spawning and therefore the post-mature non-reproductive condition in this species was related to poor nutritional status (i.e. insufficient energy reserves) (Burton & Idler 1984). Walsh *et al.* (1986) considered the non-reproductive condition of mature cod to be a result of food shortage, although no data was presented to support this hypothesis. In an experimental study, Kjesbu *et al.* (1991) found that the occurrence of atresia was inversely related to the nutritional status of the individual for Atlantic cod, a situation which reduced fecundity in poorly fed fish. This phenomenon has also been reported for three-spined stickleback, *Gasterosteus aculeatus* (Wootton 1973), rainbow trout, *Salmo gairdneri* (Scott 1962), brown trout, *Salmo trutta* (Bagenal 1969) and haddock, *Melanogrammus aeglefinus* (Hislop *et al.* 1978).

Female cod undergoing mass atresia of oocytes had significantly lower  $K_w$  than those that were ripening for the spring spawning season. However,  $K_s$  was not significantly different, indicating that the difference in  $K_w$  was due to the presence of significantly smaller ovaries and livers in the non-maturing fish, not from depletion of protein reserves in the muscle. The  $I_H$  of these fish was significantly lower than the

ripening fish. Cod store nutrients during most of the year to be used during ovarian maturation and spawning. The muscle is the main protein store while the liver is the major lipid depot (Love 1970, Eliassen & Vahl 1982, Holdway & Beamish 1985, Black & Love 1986, Kjesbu *et al.* 1991). Food deprivation experiments have been used to mimic the fasting that occurs at this time and results have shown that the first group of energy components to be broken down during starvation is usually the lipids (Black & Love 1986). Because of this, Lambert and Dutil (1997) describe cod with low  $I_H$  but normal condition as having been faced with recent (short-term) nutritional problems. Longer term, more severe malnutrition in cod is often indicated by low condition or high muscle water content.

It is somewhat difficult to explain why nearly one third of the cod in this study would elect to resorb all ripening oocytes when faced with low food availability for only a short term. Perhaps there is a nutritional critical period for gonad growth in cod, such as described for *P. americanus* (Burton 1994), whereby the attainment of a threshold level of nutrition is vital to oocyte development and failure to reach this threshold will result in interrupted development. A critical period for oocyte development in cod may exist prior to or early on in the vacuolation stage, since ovaries of cod from the Flemish Cap (Walsh *et al.* 1986) and those from Smith Sound were resorbing oocytes at this stage of development. Saborido-Rey and Junquera (1998) did not report large percentages of non-reproductive fish from Flemish Cap as opposed to the findings of Walsh *et al.* (1986). This may have been because Walsh *et al.* conducted their study of the area prior to

spawning (January-February) whereas the other study was conducted in July when spawning was complete. Perhaps mass atresia of oocytes was only visible later in the maturation cycle (nearer spawning) since it is likely that atretic oocytes do not remain in the ovary for long periods of time (Kjesbu *et al.* 1991) and resorption may have been completed by the end of the spawning period. The fact that atresia of developing oocytes appear to effect mainly those undergoing vacuolation suggests that the appearance of these oocytes may not always be a good cue to identify females that will ripen for the next spawning period, as suggested by Zamarro *et al.* (1993).

It appears that different species of fish place varying degrees of priority on the survival of the individual as compared to contributions to the survival of the population (i.e. spawning). Species such as *P. americanus* (Tyler & Dunn 1976) reportedly abandon gonad development in order to maintain condition and hopefully survive to reproduce in another year. Other species, including *Hippoglossoides platessoides* sacrifice somatic tissue to ensure that reproductive development proceeds, despite the nutritional status of the individual. Even this species will not spawn if condition is very poor (Pitt 1966). Arctic cod, *Boreogadus saida*, which is capable of spawning in multiple years, usually die after spawning due to extreme investment in oocyte production (Hop *et al.* 1995).

Survival of a fish population is dependent on successful spawning. However, it has been proposed that negative feedback occurs within a population which regulates fecundity (Oosthuizen & Daan 1974). This may apply to cod in Smith Sound, whereby if food levels are low then fecundity of some fish may be reduced to zero. Reductions in the

spawning biomass of the population means that there will be a lower recruitment into the population. This would prevent an increase in number of adult cod within the next few years and thus minimize the strain on food supply.

Fish that were undergoing mass atresia of oocytes could be divided into those that had spawned in previous years and those that had never spawned before. Those that had never spawned were categorized as adolescent. Some of the adolescent females were quite large (up to 65 cm) which may mean that attempts at ovarian maturation had been abandoned in more than one year. If the interruption of oocyte development has occurred in consecutive years, it may be possible that these females are in fact mature since the post-ovulatory follicles would not remain this long from the fish's last spawning.

Walsh *et al.* (1986) did not distinguish between resorbing fish that had spawned before and those that showed no evidence of ever spawning. Instead, all of these fish were considered post-mature non-reproductive. The present study indicates that the majority of fish classified as Spent L are in fact non-reproductive and adolescent but that this category may also contain immature and early ripening fish. Females visually assigned to the immature category contained mainly immature individuals but also some adolescent and non-reproductive fish. It is suggested that histological analysis be used when assessing the maturity of apparently non-ripening females (i.e. immature and spent L).

Ovarian wall thickness has been used to distinguish between mature and immature *P. americanus* (Burton & Idler 1984). Walsh *et al.* (1986) found a significant difference in ovarian wall thickness between immature and mature cod. Holdway and Beamish (1985),

however, indicate that at least some of the thickening of the ovarian wall in cod comes with overall growth of the fish (i.e. length). Results of the present study indicate that differences in ovarian wall thickness form immature females could be attributed to fish size. Other differences in wall thickness were due to maturity effects. Females designated as non-reproductive showed no difference in ovarian wall thickness from ripening females, indicating that ovarian wall thickness could be used to distinguish immature from non-reproductive fish. Adolescent fish had ovarian walls thicker than immature fish but thinner than ripening individuals perhaps indicating that the thickening of the wall may begin when oocytes are beginning vacuolation. Since these fish do not progress past this stage, the ovarian wall does not get as thick as those fish that do mature.

Analysis of testes revealed that male cod in Smith Sound usually have well developed gonads by January. Most individuals contained some sperm or were very near the start of spermiogenesis (sperm production). This indicates that sperm can be held, unactivated, in the testes for quite a long period, since spawning does not occur until spring. Some of the male cod examined appeared to have delayed development, with residual sperm in the central duct part of the testis, while the rest of the testis consisted of spermatogonia. Although development of gametes was delayed compared to the other males these fish were not categorized as non-reproductive. Spermatogenesis can proceed very quickly (Chapter 2) compared to oogenesis, perhaps because spermatogenesis is less energetically demanding (Karlsen *et al.* 1995), and therefore it was considered possible that the delayed males could still ripen prior to the spawning season. Perhaps another



sampling closer to spawning would have determined if these males were delayed or if they would not have taken part in spawning in 1999.

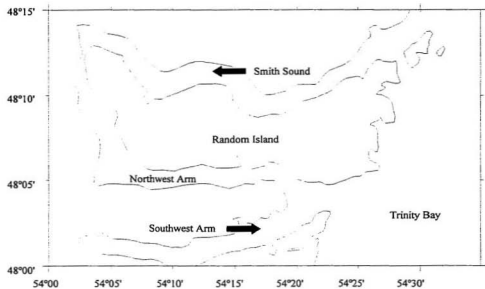


Figure 3.1. Map of eastern Newfoundland showing the position of Smith Sound.

Table 3.1. Summary of reproductive status of female cod and accuracy of visually assigned maturity stages (Non-R is non-reproductive, numbers in parentheses are percent).

Visually Assigned Maturity	Histological Maturity				TOTAL
	Immature	Ripening	Adolescent	Non-R	
Immature	47 (74.6)	0	11 (17.5)	5 (7.9)	63
Ripening	0	45	0	0	45
Spent L	3 (7.1)	9 (21.4)	7 (16.7)	23 (54.8)	42
TOTAL	50	54	18	28	150
% of total females	33.3	36.0	12.0	18.7	-

Figure 3.2. Sections of ovaries that were visually classified as Spent L. (a) Adolescent ovary with atretic oocytes (**A**) (b) Non-reproductive ovary with atretic oocytes and post-ovulatory follicles (**P**) (c) Ripening ovary (d) Immature ovary. All figures at same magnification; Scale bar = 100  $\mu$ m.

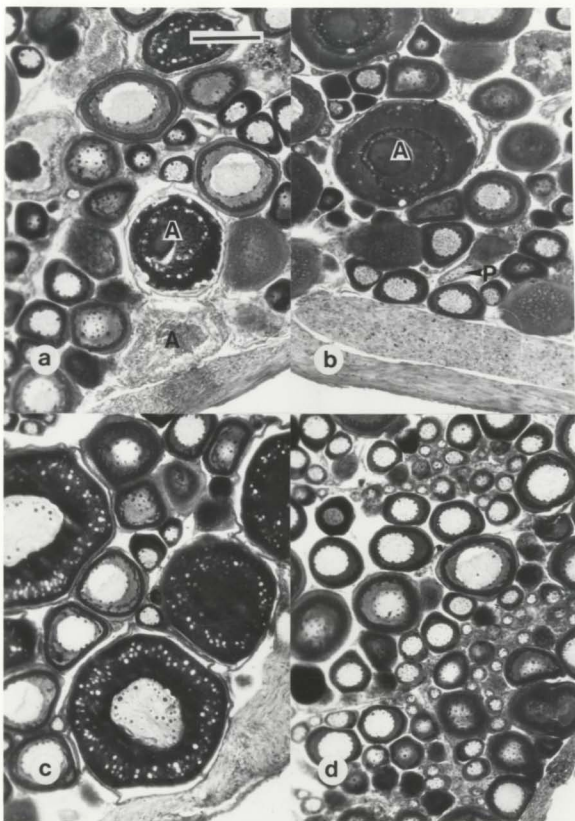


Figure 3.3. Macroscopic appearance of ovaries undergoing mass atresia of oocytes. (a) fork length 54cm,  $K_w$  1.00,  $I_G$  2.4 (b) fork length 60cm,  $K_w$  0.84,  $I_G$  1.98 (c) fork length 63cm,  $K_w$  0.83,  $I_G$  0.87.



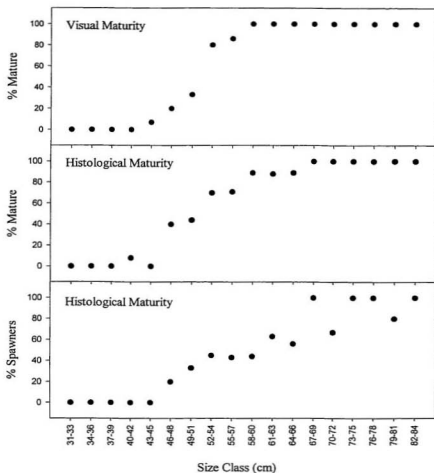


Figure 3.4. Comparison of percentage mature at size based on visual (Top) and histological (Middle) observations as well as the percentage of females in each size class that would have spawned in 1999 (Below) for female cod (n=150) from Smith Sound.



Table 3.2. Summary of various indices of condition and reproductive development of female cod from Smith Sound in January 1999. Averages are expressed as mean  $\pm$  standard deviation; ranges are indicated in parentheses.

	Maturity			
	Immature	Adolescent	Maturing	Non-R
Length (cm)	42.6 $\pm$ 5.9 (31 - 56)	51.3 $\pm$ 6.6 (43 - 65)	61.6 $\pm$ 8.5 (46 - 82)	56.2 $\pm$ 7.8 (42 - 79)
$K_w$	0.923 $\pm$ 0.074 (0.769 - 1.086)	0.897 $\pm$ 0.082 (0.768 - 1.068)	0.936 $\pm$ 0.077 (0.768 - 1.104)	0.856 $\pm$ 0.075 (0.727 - 1.007)
$K_s$	0.830 $\pm$ 0.064 (0.685 - 0.980)	0.804 $\pm$ 0.076 (0.697 - 0.933)	0.803 $\pm$ 0.058 (0.684 - 0.922)	0.772 $\pm$ 0.059 (0.692 - 0.870)
$I_o$	0.386 $\pm$ 0.136 (0.078 - 0.748)	0.599 $\pm$ 0.199 (0.247 - 1.011)	3.177 $\pm$ 1.409 (1.387 - 8.364)	1.281 $\pm$ 0.558 (0.688 - 2.449)
$I_H$	4.053 $\pm$ 1.130 (1.719 - 6.053)	4.125 $\pm$ 1.184 (2.462 - 6.374)	5.326 $\pm$ 0.986 (3.333 - 7.425)	3.782 $\pm$ 1.447 (1.655 - 6.326)
Wall ( $\mu$ m)	44.4 $\pm$ 11.9 (25 - 63)	69.3 $\pm$ 20.1 (46 - 107)	167 $\pm$ 57.9 (83 - 312)	135.9 $\pm$ 47.0 (107 - 265)

Table 3.3. Summary of regression analyses to determine the relationship between fish length and  $K_w$ ,  $K_s$ ,  $I_a$ ,  $I_H$  and ovarian wall thickness for female cod from Smith Sound in January (significance level of  $\alpha=0.05$ ).

Maturity	$K_w$			$K_s$			$I_a$			$I_H$			Ovarian Wall		
	$r^2$	df	p	$r^2$	df	p	$r^2$	df	p	$r^2$	df	p	$r^2$	df	p
Immature	21.4	49	0.001	20.6	48	0.001	0.7	49	0.557	1.5	48	0.405	27.9	19	0.017
Adolescent	21.5	17	0.052	26.8	13	0.058	21.6	17	0.052	0.9	13	0.794	3.8	14	0.485
Ripening	0.5	43	0.658	0.0	43	0.988	0.1	43	0.874	0.2	43	0.758	21.7	14	0.080
Non-reproductive	6.1	26	0.213	30.9	8	0.120	6.7	26	0.191	8.0	8	0.460	12.3	21	0.110

Table 3.4. *P*-values from Analysis of Covariance of  $K_w$ ,  $K_s$ ,  $I_G$ ,  $I_H$  and ovarian wall thickness between the immature, adolescent, ripening and non-reproductive female cod collected in Smith Sound in January 1999 (covariate=length;  $\alpha=0.05$ ).

Comparison	$K_w$	$K_s$	$I_G$	$I_H$	Ovarian Wall
Imm. vs. Rip.	-	-	<0.001	0.000	0.002
Imm. vs. Ad.	0.266	0.430	-	0.536	0.029
Imm. vs. NR	0.850	0.606	<0.001	0.956	0.006
Ad. vs. Rip.	0.037	-	<0.001	0.002	0.001
Ad. vs. NR	0.457	0.396	<0.001	0.611	0.002
Rip. vs. NR	<0.001	0.156	<0.001	0.001	0.525

Table 3.5. Summary of reproductive status of male cod and accuracy of visually assigned maturity stages (numbers in parentheses represent percent).

Visually Assigned Maturity	Histological Maturity			TOTAL
	Immature	Ripening	Delayed	
Immature	23 (88.5)	1 (3.8)	2 (7.7)	26
Ripening	0	83 (93.3)	6 (6.7)	89
Spent L	1 (9.1)	6 (54.5)	4 (36.4)	11
TOTAL	24	90	12	126
% of total males	19.0	71.4	6.5	-

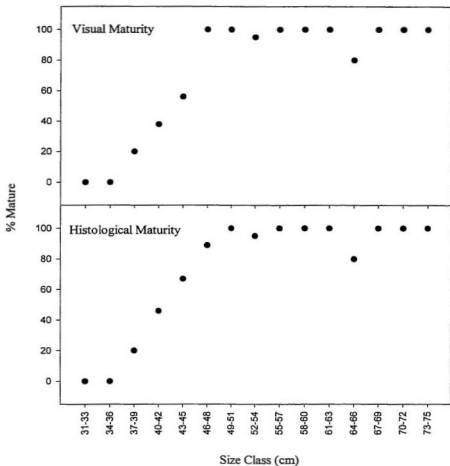


Figure 3.5. Comparison of percentage mature at size based on visual (Top) and histological (Below) observations for male cod ( $n=126$ ) from Smith Sound in January 1999.

Figure 3.6. Sections of testes collected from Atlantic cod in Smith Sound in January. (a) Immature male (b) Delayed male with residual spermatozoa (**RS**) (c) Ripening male with no sperm present (d) Ripening male with sperm present (**S**). All figures at same magnification; Scale bar = 50  $\mu\text{m}$ .

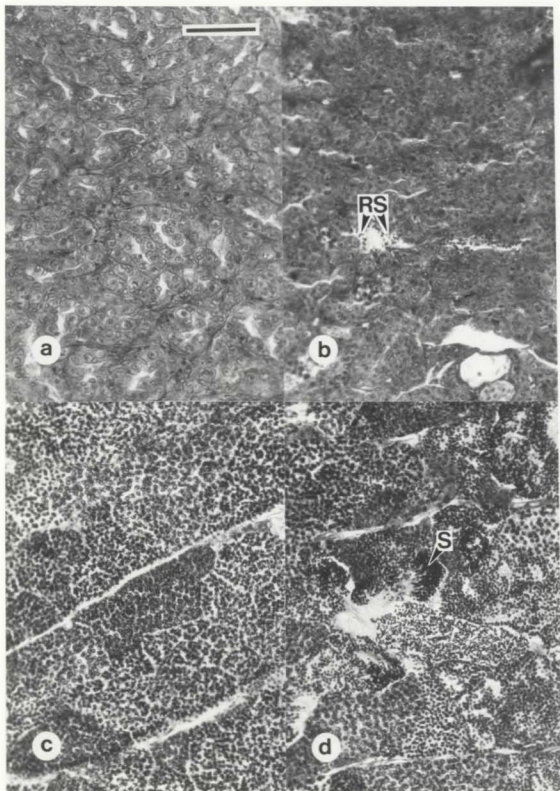


Table 3.6. Summary of indices of condition and reproductive status of male cod from Smith Sound in January 1999. Averages are expressed as mean  $\pm$  standard deviation; ranges are indicated in parentheses.

	Maturity			
	Immature	Ripening (sperm absent)	Ripening (sperm present)	Delayed
Length (cm)	42.0 $\pm$ 7.0 (32-66)	54.2 $\pm$ 8.6 (37-68)	54.6 $\pm$ 7.5 (42-74)	49.8 $\pm$ 8.0 (42-64)
$K_w$	0.894 $\pm$ 0.067 (0.754-1.016)	0.911 $\pm$ 0.074 (0.775-1.068)	0.954 $\pm$ 0.073 (0.759-1.121)	0.876 $\pm$ 0.074 (0.754-1.012)
$K_s$	0.808 $\pm$ 0.061 (0.682-0.911)	0.791 $\pm$ 0.064 (0.664-0.907)	0.808 $\pm$ 0.062 (0.674-0.968)	0.783 $\pm$ 0.062 (0.706-0.893)
$I_G$	0.173 $\pm$ 0.071 (0.075-0.357)	3.009 $\pm$ 2.021 (0.147-8.817)	6.139 $\pm$ 3.116 (0.221-12.870)	0.758 $\pm$ 0.764 (0.135-2.568)
$I_H$	3.492 $\pm$ 1.055 (1.429-5.333)	4.042 $\pm$ 0.651 (2.887-5.263)	4.264 $\pm$ 1.036 (1.290-6.477)	5.013 $\pm$ 1.235 (2.906-6.667)



Table 3.7. Summary of regression analyses to determine the relationship between fish length and  $K_w$ ,  $K_s$ ,  $I_0$  and  $I_H$  for male cod from Smith Sound in January (significance level of  $\alpha=0.05$ ).

Maturity	$K_w$			$K_s$			$I_0$			$I_H$		
	$r^2$	df	p	$r^2$	df	p	$r^2$	df	p	$r^2$	df	p
Immature	0.2	25	0.843	0.1	24	0.860	66.6	25	<0.001	5.6	24	0.255
Ripening (no sperm)	3.5	8	0.631	21.4	7	0.248	56.4	8	0.020	0.1	7	0.935
Ripening (sperm)	0.1	57	0.852	1.7	51	0.362	5.1	57	0.089	7.2	51	0.055
Delayed	7.1	18	0.271	0.5	16	0.792	14.0	18	0.115	36.8	16	0.010

Table 3.8.  $P$ -values from Analysis of Covariance of  $K_w$ ,  $K_s$ ,  $I_G$  and  $I_H$  between the immature, ripening (no sperm), ripening (sperm) and delayed male cod collected in Smith Sound in January 1999 (covariate=length,  $\alpha=0.05$ ).

Comparison	$K_w$	$K_s$	$I_G$	$I_H$
Imm. vs. Rip. (no sperm)	0.126	0.867	<0.001	0.391
Imm. vs. Rip. (sperm)	0.020	0.981	<0.001	0.930
Imm. vs. Delayed	0.946	0.589	-	0.017
Rip. (no sperm) vs. Rip. (sperm)	0.015	0.293	<0.001	-
Rip. (no sperm) vs. Delayed	0.056	0.377	0.002	0.016
Rip. (sperm) vs. Delayed	0.002	0.261	<0.001	0.006

-significant interaction term.

## CHAPTER 4

### **Peculiarities in ovarian structure leading to multiple year delays in oogenesis and possible senescence in Atlantic cod, *Gadus morhua* L.**

#### **4.1 ABSTRACT**

Four specimens of female Atlantic cod, *Gadus morhua* L., collected between 1997 and 1999 had ovarian structures which suggested multiple year interruptions in the spawning cycle of these fish was occurring. One specimen was experiencing a minimal two year delay in its reproductive cycle because the ovary contained only oogonia, which would have had to mature before any spawning could occur. Two other specimens did not contain oogonia or perinucleolar stage oocytes, suggesting that these ovaries were senescent. The final ovary, removed from a broodstock female contained large masses of reabsorbing hyaline oocytes which would likely have impaired the normal release of hydrated oocytes in the next spawning season.

## 4.2 INTRODUCTION

The phenomenon of non-annual spawning has been reported for Atlantic cod, *Gadus morhua* L., from inshore (Chapter 3) and offshore (Walsh *et al.* 1986) areas in the northwest Atlantic as well as the Barents Sea (Oganesyan 1993). Spawning omission has also been noted for Greenland halibut, *Reinhardtius hippoglossoides* (Fedorov 1968, 1971), winter flounder, *Pleuronectes americanus* (Burton & Idler 1984) and American plaice, *Hippoglossoides platessoides* (Pitt 1966) and may be a normal event in the life history of many long-lived teleosts. The non-reproductive nature of fish that have spawned in previous years has been attributed to adverse environmental conditions (Woodhead & Woodhead 1965, Fedorov 1971) or low condition as a result of inadequate food supply (Walsh *et al.* 1986, Oganesyan 1993, Burton & Idler 1984).

The reported interruptions in the spawning cycle of cod and other species are assumed to be one year delays and the fish can spawn successfully in the following year if conditions are suitable (Burton 1991; Maddock 1997). Reports of long-term interruptions (i.e. multiple years) are very scarce. The only such report for Atlantic cod pertains to the account of ovarian stromal fibrosis observed in four individuals from the northwest Atlantic (Wiles 1969). The following study adds to the observations of long-term interruptions in the spawning cycle of Atlantic cod and the possibility of senescence among some individuals.

### **4.3 MATERIALS AND METHODS**

Three abnormal ovaries were collected during regular sampling of Atlantic cod from around Newfoundland and Labrador. Specimen 1 was collected in December, 1998 from Placentia Bay by gill net. Specimens 2 and 3 were collected by bottom trawl in January, 1999 from NAFO area 2J (off southern Labrador) and Smith Sound, Trinity Bay, respectively. A fourth ovary was collected from a laboratory-held Atlantic cod that was killed in December, 1997 at the Ocean Sciences Centre, Logy Bay, Newfoundland. Ovaries were fixed in Bouin's solution and processed, sectioned and stained as per Chapter 1.

#### 4.4 RESULTS

The size and condition of the four fish is presented in Table 4.1. All the fish were of good condition and specimens 1,3, and 4 were likely mature, if length is used as an indicator. Specimen 2 was smaller and may have been immature.

Ovaries from specimens 1-3 appeared healthy macroscopically, being very small and light to dark pink giving the appearance of an immature or spent ovary. Histologically, specimens 1 and 2 appeared similar in that the only observable oocytes appeared to be degenerating (Fig 4.1a). These oocytes were slightly eosinophilic and had what appeared to be the remains of follicles surrounding them. There were no oogonia (Fig 4.1b) and no perinucleolar stage oocytes to form the basis for future spawning. Specimen 3 did not contain any large degenerating oocytes. It contained only scattered areas of a few small perinucleolar stage oocytes (Fig 4.1c) and the rest of the ovarian tissue appeared to be made up of many thousands of oogonia (Fig 4.1d).

The ovary of specimen 4, unlike the other three ovaries, did not appear normal macroscopically. The posterior portion of the ovaries appeared normal while the anterior regions were shrivelled with a hard, bumpy texture (Fig. 4.2). Over half of the left ovary had this abnormal appearance while only the posterior third of the right ovary appeared this way. Transverse sections of the ovary revealed areas of hard, brown, shiny matter clumped together in various areas throughout the ovary. Histology revealed these areas to be clusters of resorbing hyaline oocytes. Only a few of these areas existed in the posterior part of the ovaries (Fig 4.3a) while the anterior portions were virtually filled with large

aggregations of hyaline oocytes (Fig 4.3b). Throughout the ovaries there were also oocytes in the stage of cortical alveoli accumulation.

## 4.5 DISCUSSION

The purpose of the present study is simply to add to the knowledge of the total possibilities for reproductive status of individual Atlantic cod and to confirm the possibility of long-term interruptions in the reproductive cycle. It is hypothesised that all four fish would have experienced multiple year delays in oogenesis.

Specimen 3 contained a very unsubstantial number of oocytes in the protoplasmic growth phase with the rest of the ovary being filled with many thousands of oögonia. The amount of spawning that could have been accomplished by the ripening of the presently immature oocytes probably would have been negligible since they were so few in number. Any significant amount of spawning in the future would have had to be accomplished by the conversion of oögonia into oocytes and their subsequent ripening. Very few estimates of oocyte development time have been published. It has been estimated that total oocyte maturation time (oögonium to hyaline oocyte) in the winter flounder, *Pleuronectes americanus*, is three years (Dunn & Tyler 1969; Dunn 1970; Burton & Idler 1984). Farmed Atlantic cod from coastal Norway (Karlsen *et al.* 1995) and female cod in the more southern stocks in the northwest Atlantic (Trippel 1995), however, often spawn at two years of age, suggesting a minimum period of 2 years for the development from oögonia to mature oocyte in this species.. This suggests that the delay in the reproductive cycle for specimen 3 would have been at least 2 years but may have been much longer since oögonia could have remained in a 'resting' state (i.e. not transformed into oocytes) for many years.



The lack of oogonia and perinucleolar stage oocytes in specimens 1 and 2 seems to indicate a complete abandonment of reproduction (i.e. senescence) for future years since there was nothing to form the basis for future oocyte production. The presence of degenerating oocytes may indicate that the fish were ripening oocytes before resorption began.

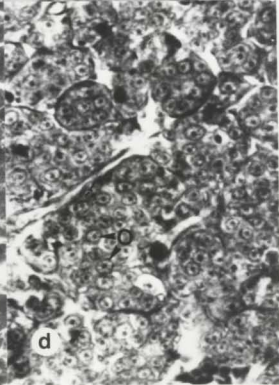
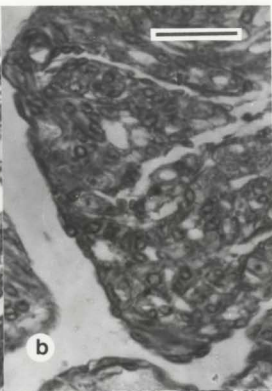
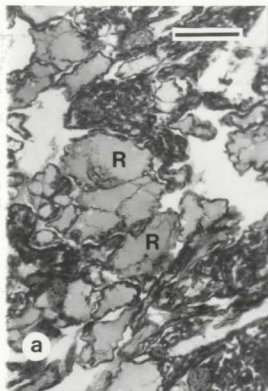
The ovaries of specimen 4 appeared deformed, with the anterior part of both ovaries appearing shrivelled with a hard, bumpy surface. Clumps of hard brown material could be seen in cross-sections of the ovaries and histological examination revealed this to be masses of reabsorbing hyaline oocytes. The masses were small and few in the posterior part of the ovaries which appeared normal with visual inspection but were the predominant feature of the anterior ovary. Despite the fact that oocytes throughout the ovary were undergoing vacuolation it is predicted that the spawning of any mature oocytes would have been impaired by the plugs of hyaline oocytes, especially in the anterior region of the ovaries. Spawning may have proceeded normally in the posterior portion of the ovaries, however, records on individual fish do not show any spawning activity for this female in the previous year, likely indicating a long-term interruption in the spawning cycle.

The present study shows that multiple year interruptions in spawning cycles can occur for Atlantic cod, *Gadus morhua*, although the causes of such interruptions are unknown and that ovarian senescence can occur in relatively small fish.

Table 4.1. Data on the size and condition of four Atlantic cod with abnormal ovaries.

Specimen	Length (cm)	Weight (g)	$K_W$	$I_G$
1	59	2004	0.976	0.649
2	39	520	0.877	0.192
3	59	2175	1.059	0.092
4	77	4558	0.998	1.996

Figure 4.1. Sections of Atlantic cod ovaries which appeared normal visually but had an abnormal microscopic appearance. (a) Senescent ovary with degenerating oocytes (**R**) and (b) complete lack of oogonia (c) Ovary with small groups of perinucleolar stage oocytes (**P**) and (b) huge numbers of oogonia (**O**). Figures (a) and (c) at same magnification; Scale bar = 100  $\mu\text{m}$ . Figures (b) and (d) at same magnification; Scale bar = 50  $\mu\text{m}$ .



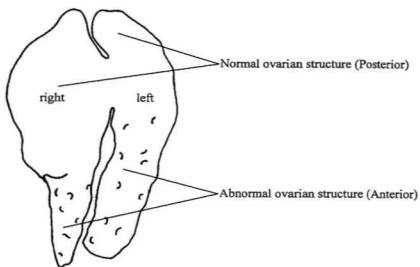
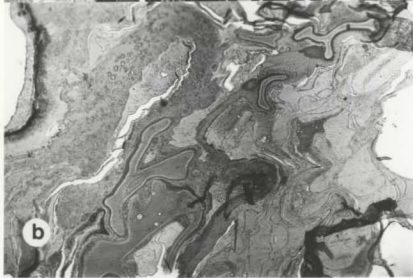
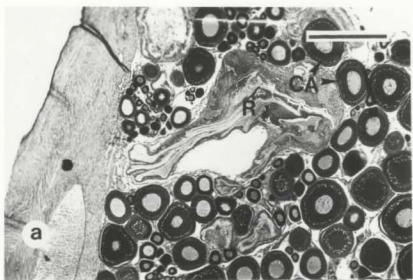


Figure 4.2. Macroscopic appearance of abnormal ovary removed from captive Atlantic cod in December, 1997.

Figure 4.3. Sections of the abnormal ovary (see Figure 4.2) removed from a broodstock female (a) Small area of resorbing hyaline oocytes (**R**) and cortical alveoli stage oocytes (**CA**) in the posterior part of the ovary and (b) large area of resorbing hyaline oocytes in the anterior part of the ovary. Both figures at same magnification; Scale bar = 500  $\mu\text{m}$ .



## CONCLUSIONS

Atlantic cod, *Gadus morhua* L., spawn both offshore and inshore in the waters around Newfoundland and Labrador. Cod in Placentia Bay spawned throughout the spring and summer in 1998. Some fall spawning may also have taken place. Female cod began to ripen oocytes early in winter and oocytes and ovaries continued to increase in size up until spawning. During the winter protein stored in the white muscle was mobilised for respiration and gonad growth. Male cod began gamete development in the fall and completed spermatogenesis before the winter reduction in feeding. Therefore, gamete development in males was, at least partially, fuelled by the energy in the food consumed during the fall. White muscle protein stores were not affected during testicular growth but were utilized for body maintenance functions during the winter. The commencement of feeding in the spring replenished muscle protein stores in both male and female cod.

The fecundity of Atlantic cod was usually determined long before spawning indicating that the spawning pattern was determinate. Mechanisms existed, however, to alter fecundity such that the number of vitellogenic oocytes present in the ovary before spawning may not have been an accurate estimate of the number of oocytes that would have been spawned. Atresia of both previtellogenic and vitellogenic oocytes downgraded fecundity and may have been a result of low condition from a poor feeding season. In rare cases fecundity was boosted by *de novo* vitellogenesis during spawning. This additional recruitment of oocytes into vitellogenesis may have occurred only in fish that were still in



very good condition after the winter. These observations were not consistent with the definition of a determinate spawner. It appears that cod were capable of a form of indeterminate spawning if conditions were really good. Otherwise, maximum fecundity was determined several months prior to spawning and could be downgraded by follicular atresia if the environment or the condition of the fish deteriorated.

The spawning periodicity of Atlantic cod in the inshore environment may be dependent on environmental conditions including food availability. Rare cases of mass atresia of vitellogenic oocytes occurred in Placentia Bay cod in 1998, suggesting that spawning was omitted by some individuals. Approximately one third of the female cod collected from Smith Sound in January were undergoing mass atresia of oocytes that had begun to ripen and therefore would not have spawned in 1999. These fish included females that had started ripening for the first time and some that had spawned in previous years. The ovarian walls of the females that had spawned previously were thicker than those that had not and could be used to distinguish mature from immature fish. Liver indices were lower for fish with resorbing oocytes than females that were ripening but somatic condition did not differ, perhaps suggesting recent food shortage during some critical period was responsible for the reversal in gonad development. Interruptions of the spawning cycle such as this are believed to last for only one year. A small percentage of males from Smith Sound had testes that showed no signs of ripening in January but may still have ripened in time to take part in spawning.

Multiple year interruptions in the spawning cycle occurred in a very small number

of female cod from different areas of the Northwest Atlantic. These included ovaries with mainly oögonia which would have taken a minimum of two years to mature and ovaries with physical barriers to the release of mature oocytes caused by inefficient expulsion of last year's hyaline oocytes from the ovary. Rare cases of senescent ovaries also existed in which there were no oögonia or immature oocytes to form the basis for future spawning episodes.

Gonad development in cod is not independent of the environment. Fecundity can be increased when food is abundant and the fish is in good condition. When conditions are not as favourable, fecundity can be lowered by follicular atresia and reproductive development can be reversed by the mass resorption of all ripening oocytes causing the fish to skip a spawning season. Abnormal ovarian structure, for which the cause is unknown, can lead to multiple year delays in the reproductive cycle.

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